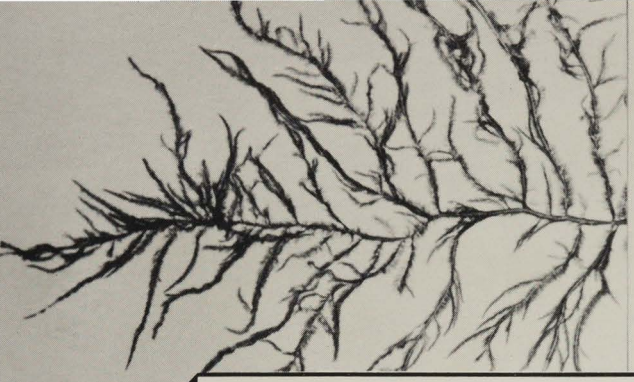
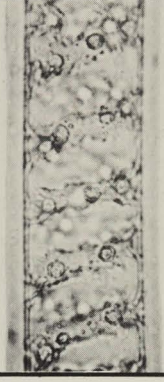

PHYCOLOGICAL STUDIES

VII. Taxonomic Investigations of Stigeoclonium

ELENOR R. COX AND HAROLD C. BOLD

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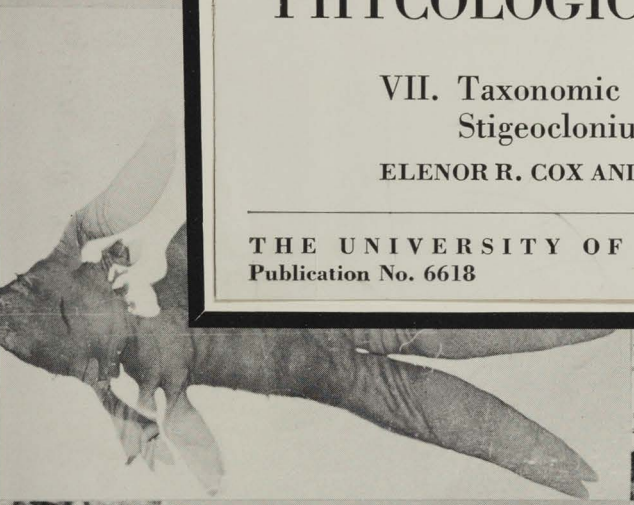
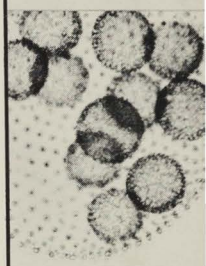
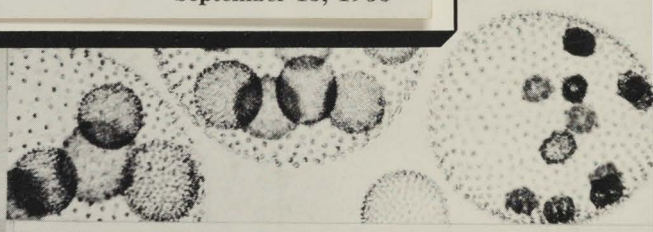
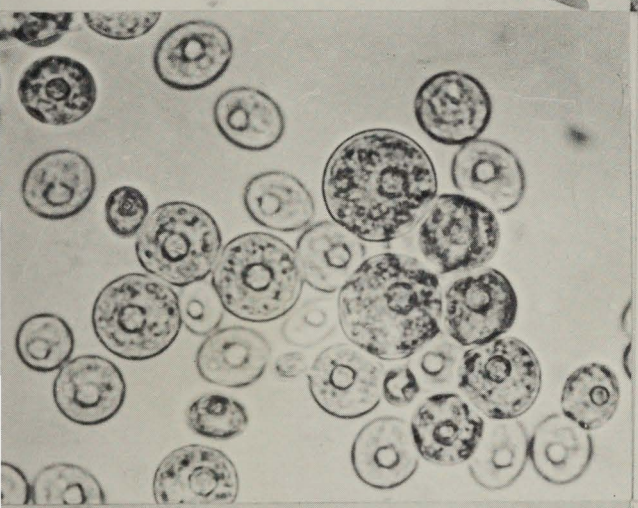

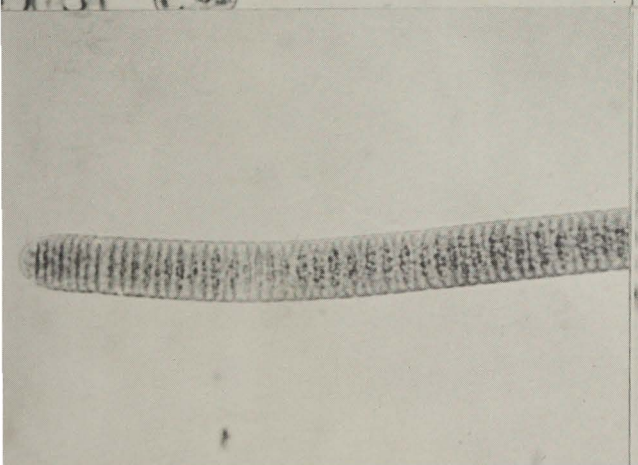
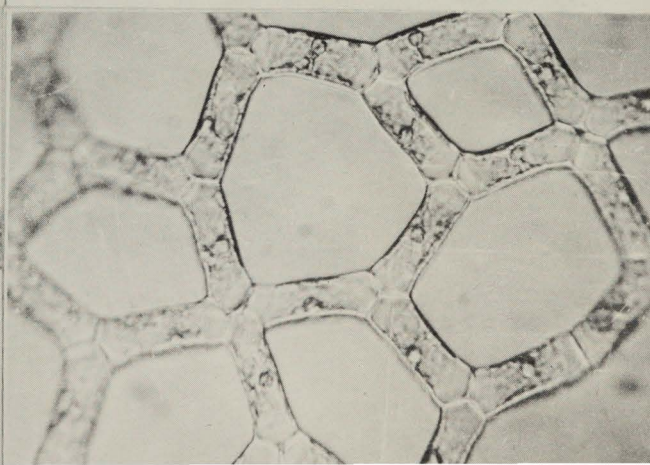

Phycological Studies

VII. Taxonomic Investigations of Stigeoclonium

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Introduction

The algal genus *Stigeoclonium* Kützing (Order Ulotrichales,[1] Family Chaetophoraceae) includes all branched, uniseriate, filamentous Chlorophyceae in which the cells of the main axis and the branches are similar in size. The plant is usually differentiated into a prostrate system, which anchors or attaches it to some substrate, and a branched, erect system which develops from the cells of the prostrate system.

The genus *Stigeoclonium,* described almost 125 years ago, constitutes a well-known and, for the most part, clearly defined taxonomic unit. It is usually not difficult to distinguish a *Stigeoclonium,* in nature or in the laboratory, from members of closely allied genera such as *Cloniophora, Draparnaldia,* or *Chaetophora*—although inevitably a few overlapping forms exist. However, in contrast to the relative ease of generic determination, specific identification—at present based largely or exclusively on the vegetative characters of the erect system—is extremely difficult. Attempts of previous workers, through comparative studies of preserved materials, or through reinterpretation of written descriptions, have done little to clarify—and sometimes much to confuse—the situation.

A primary objective of this study has been to determine which attributes of the erect system are reliable taxonomic criteria, and which are not. Toward this end, comparative cultural studies were conducted under controlled conditions to establish the range of variation of each trait in a single organism. In addition, the morphology of the basal system was studied intensively in order to determine the taxonomic value of this attribute.

A second objective of this study has been to determine whether the genus *Caespitella,* as defined by Vischer (1933), and subsequently ignored by other workers, is distinct enough to warrant its retention, or whether it should be combined with *Stigeoclonium.* Toward this end, three isolates corresponding to Vischer's description of *Caespitella pascheri* were included in this investigation.

In the opinion of the writers, the approach adopted in this study provides a reliable basis for future studies of the genus *Stigeoclonium.* The authors are well aware that increased emphasis on the culture method, and on morphological attributes which are not immediately visible in collected materials, will not please those who seek to simplify the identification of *Stigeoclonium* in the field. However, if it were now possible to identify species of *Stigeoclonium* easily and reliably in the field, this investigation would not have been necessary.

Materials and Methods

A preliminary study was undertaken of 80 branched, filamentous, "*Stigeoclonium*-like" algal isolates obtained principally as epiphytes on aquatic angio-

[1] Smith, 1950.

sperms growing in the freshwater streams in and near Austin, Texas. Cultures from other collectors and from the Culture Collection of Algae at Indiana University were added to these 80 isolates. Sixty of these organisms were obtained in axenic culture and after a careful consideration of these, the 20 which represented the most diverse characteristics were chosen for intensive study. The sources of the 20 isolates used during the course of this investigation are shown in Table 1.

Unialgal cultures of the algae growing as epiphytes on aquatic vegetation were obtained in the following manner. A few leaves of the plant were placed in a Pyrex Petri dish (100 × 20 mm) in a medium consisting of 3 parts Modified Bold's Basal Medium (BBMP) and 1 part filtered soil supernatant.[2] From 1 to 2 weeks later, the vegetation was removed and the algae growing on the bottom of the Petri dish were examined with a stereoscopic binocular microscope. In this manner, both the erect filaments and the basal systems of the algae could be examined before isolations were made. Isolations were made by detaching portions of the erect filaments with a fine platinum needle and carefully pulling the filaments over and through a 1% agar surface with the needle, after which the filaments were rinsed through several drops of distilled water. In this manner the plants could usually be freed of algal contaminants. A single filament was then placed in a bi-phasic soil-water tube for growth and maintenance. Each of the cultures used in this study originated from the isolation of a single filament. After a unialgal culture had been established in bi-phasic soil tubes, transfers were often made to tubes containing BBMP or 3 parts BBMP plus 1 part soil supernatant.

Axenic cultures were obtained from the unialgal isolates as follows. Actively growing unialgal cultures were placed in a diSONtegrator (system 80—Ultrasonic Industries, Inc.) in order to break the filaments into smaller pieces. The amount of time required for fragmentation varied with the isolate. Most of the isolates required from 1 to 2 min. The algae were then centrifuged and the supernatant decanted, after which they were allowed to stand from 2 to 6 hr in a 4% solution of Tween-80 (Atlas Powder Company, Wilmington, Delaware). The algae were then centrifuged, washed with sterile distilled water, sonicated, and again centrifuged. This process, which proved to be the most essential step in obtaining bacteria-free cultures, was repeated from 10 to 15 times. A small drop of the algal suspension was pipetted aseptically into the bottom of a previously sterilized Pyrex Petri dish (100 × 20 mm) and cool 2% BBMP agar was poured into the plate. The plate was swirled to disperse the algal filaments evenly throughout the agar medium. After an incubation period of 1–2 weeks in a culture chamber at 22° C and in a 12/12-hr light and dark cycle, bacteria-free filaments could usually be picked up from the surface of the agar with a fine platinum needle. These filaments were transferred to slants of proteose-peptone agar for growth and maintenance.

[2] Supernatant of a steamed soil-water bottle prepared according to the method of Pringsheim (1946); see p. 10.

TABLE 1. *Sources and isolators of organisms investigated*

Organism	Code[a]	Source of the Isolate	Isolator
S. helveticum	S–4	I.U. 441[b] (as *S. helveticum* var. *maius*)	Vischer
S. aestivale	Var 5	Cleaning Pond, Waste Stabilization Pond, Austin, Texas	Cox
S. aestivale	HP 4	Waller Creek at E. 38th Street, Austin, Texas	Cox
S. aestivale	8–3	Epiphytic on *Elodea*, Biology Pond, University of Texas, Austin, Texas	Cox
S. subsecundum	19–11–V	Epiphytic on *Vallisneria*, Landa Park, New Braunfels, Texas	Cox
S. tenue	6–1D	Epiphytic on *Vallisneria*, San Marcos, Texas	Cox
S. tenue	Var 1	Cleaning Pond, Waste Stabilization Pond, Austin, Texas	Cox
S. tenue	Gold	Intestinal tract of goldfish	Cox
S. tenue	19–1–E	Epiphytic on elm rootlets, Landa Park, New Braunfels, Texas	Cox
S. pascheri	Ca 421	I.U. 421[b] (as *Caespitella* sp.)	Lewin
S. pascheri	10–2	Waller Creek at E. 38th Street, Austin, Texas	Cox
S. pascheri	18–3	Epiphytic on *Hydrocotyle*, Landa Park, New Braunfels, Texas	Cox
S. variabile	6–15	Epiphytic on *Vallisneria*, San Marcos, Texas	Cox
S. variabile	6–23	Epiphytic on *Vallisneria*, San Marcos, Texas	Cox
S. variabile	Jo	Epiphytic on vegetation, Hamilton Pool, near Austin, Texas	Cox
S. farctum	19–5–V	Epiphytic on *Vallisneria*, Landa Park, New Braunfels, Texas	Cox
S. farctum	5–3C	Epiphytic on vegetation, Florida	Cox
S. farctum	5–3F	Epiphytic on vegetation, Florida	Cox
S. farctum	7–17	Epiphytic on *Ceratophyllum*, San Marcos, Texas	Cox
S. sp.	S–5	I.U. LB 439[b] (as *S. farctum* Berthold)	Butcher

[a] Writers' isolation numbers.

[b] Culture collection of algae, Indiana University, Bloomington, Indiana (Starr, 1964).

The following media were used for isolation, preliminary growth, and maintenance of cultures:

1. Bi-phasic soil-water tubes, prepared as follow:
   Less than 0.1 g $CaCO_3$ was placed in the bottom of a small test tube (13 × 100 mm) and covered with approximately 1 g of sifted soil.[3] Enough water was added to fill the tube 2/3 full, and the tube was autoclaved 15–20 min at 15 lb pressure. The tubes were allowed to settle about 48 hr before use.
2. Modified Bold's Basal Medium (BBMP),[4] prepared as follows:
   To 940 ml of deionized or glass-distilled water were added 10 ml of each of six stock solutions. The concentration of each of the stock solutions is listed below:

	g/1000 ml	*Percent*	M*/1000 ml*
$NaNO_3$	25.0 g	2.50	0.30 M
KH_2PO_4	15.0 g	1.50	0.10 M
K_2HPO_4	10.0 g	1.00	0.06 M
$MgSO_4 \cdot 7H_2O$	7.5 g	0.75	0.03 M
$CaCl_2 \cdot 2H_2O$	2.5 g	0.25	0.02 M
NaCl	2.5 g	0.25	0.04 M

To this was added 1 ml of each of the following minor element stock solutions:

EDTA Stock Solution
50 g EDTA (Ethylenediaminetetraacetic Acid) and 31 g KOH are diluted to 1 liter with deionized or glass-distilled water.

H-Fe Stock Solution
4.98 g $FeSO_4 \cdot 7H_2O$ are dissolved in 1 liter of acidified water. The latter is made by adding 1 ml concentrated H_2SO_4 to 999 ml deionized or glass-distilled water.

H-Boron Stock Solution
11.42 g H_3BO_3 are dissolved in 1 liter of deionized or glass-distilled water.

$H\text{-}H_5$ Stock Solution
8.82 g $ZnSO_4 \cdot 7H_2O$
1.44 g $MnCl_2 \cdot 4H_2O$
0.71 g MoO_3
1.57 g $CuSO_4 \cdot 5H_2O$
0.49 g $Co(NO_3)_2 \cdot 6H_2O$.

[3] Soil from a garden in Nashville, Tennessee.

[4] The concentration of phosphates differs slightly from that used by Cain (1963) and Bischoff (1963). In their work the concentraion of KH_2PO_4 and K_2HPO_4 was 1.75% and 0.75% respectively. Wiedeman (1964) designated the medium which is outlined above as Modified Bold's Basal Medium (BBM2). Although the two media give comparable growth results, the abbreviation BBMP will be used throughout this work to designate the difference in the phosphate concentrations.

These are dissolved in 1 liter of acidified water (prepared as above).

BBMP has a pH of 6.3–6.6 after autoclaving and cooling. The concentration per liter of each of the six salts in the final medium is as follows:

$NaNO_3 = 3.0$ mM; $KH_2PO_4 = 1.0$ mM; $K_2HPO_4 = 0.6$ mM; $MgSO_4 \cdot 7H_2O = 0.3$ mM; $CaCl_2 \cdot 2H_2O = 0.2$ mM; and $NaCl = 0.4$ mM.

3. BBMP supplemented with soil extract, *organic*, prepared as follows:
200 g of soil and 2 g $CaCO_3$ were autoclaved for 30 min in a liter of deionized or glass-distilled water. The solution was allowed to settle for several days, after which the supernatant was filtered and combined with BBMP (3 parts BBMP: 1 part soil supernatant) before using.

4. Proteose-peptone agar slants, prepared as follows:
1 g of proteose-peptone (Difco Laboratories, Detroit, Michigan) was added to 1 liter of BBMP, and the solution was solidified with 15 g Difco Bacto-Agar (Difco Laboratories, Detroit, Michigan).

The following media were used routinely throughout the course of this investigation to test cultures for the presence of bacteria:

1. Yeast Extract Agar, prepared as follows: 0.5 g of yeast extract (Baltimore Biological Laboratory, Baltimore, Md.) was added to 1 liter of BBMP and the solution solidified with 15 g of Difco Bacto-Agar.

2. Nutrient Agar (Difco Laboratories, Detroit, Michigan).

3. Nutrient Broth (Difco Laboratories, Detroit, Michigan).

4. Thioglycollate Broth (Difco Laboratories, Detroit, Michigan).

The media listed as 2, 3, and 4 above were prepared as directed by Difco.

Since a comparative study of the vegetative morphology of *Stigeoclonium* and *Caespitella*-like organisms, as grown in laboratory culture, forms an integral part of this study, much time was spent devising a defined culture medium in which the isolates not only grew well, but one in which the morphology of the organisms would be "normal."[5] From preliminary experiments it was evident that most of the isolates grew better in a mixture of BBMP plus soil supernatant (3:1) than in BBMP alone. Later experiments showed that the morphology of the *Stigeoclonium* isolates in culture approximated the morphology of the isolates grown in nature and in the undefined BBMP supplemented with soil supernatant (3:1) when the pH of BBMP was raised to 7.5–7.6 by the addition of tris-(Hydroxymethyl)

[5] The writers hesitate to use this term because in the absence of some standard which is known to be "normal," its meaning is too subjective for precise interpretation. The term "normal growth" is here defined to mean the expression of that morphological characteristic or group of characteristics most often seen in an undefined and enriched medium such as BBMP and soil supernatant (3:1) or in nature.

Aminomethane.[6] Thus, BBMP buffered with TRIS to a pH of 7.5–7.6 and enriched with cyanocobalamin (Vitamin B_{12})[7] was used as the standard culture medium in which morphological observations were made.

This medium, designated as BBMPTB$_{12}$, was prepared as follows:

	Stock Soln.	*For 1 liter use*	*Final Conc. (per liter)*
$NaNO_3$	0.1 M (4.25 g/500 ml)	30 ml	3.0 mM
KH_2PO_4	0.1 M (6.805 g/500 ml)	10 ml	1.0 mM
K_2HPO_4	0.1 M (8.71 g/500 ml)	6 ml	0.6 mM
$MgSO_4 \cdot 7H_2O$	0.1 M (12.325 g/500 ml)	3 ml	0.3 mM
$CaCl_2 \cdot 2H_2O$	0.1 M (7.351 g/500 ml)	2 ml	0.2 mM
NaCl	0.1 M (2.925 g/500 ml)	4 ml	0.4 mM
TRIS Buffer	0.2 M (12.114 g/500 ml)	25 ml	0.5 mM
B_{12}	10 γ/ml	2 ml	20 γ

One milliliter of each of the four minor element solutions for BBMP[8] was added to each liter of medium. The pH of the medium was adjusted with ~ 1 N HCl; 2 ml of ~ 1 N HCl gave a final pH of 7.5–7.6 after autoclaving and cooling. All of the compounds used in making stock solutions were weighed on a Mettler Balance, Type H16.

Axenic stock cultures were maintained on BBMPTB$_{12}$ agar slants in which the concentration of $NaNO_3$ was 6.0 mM or 9.0 mM per liter.

All experiments were conducted in a culture chamber under controlled environmental conditions, as follows: 22°C, incident light of approximately 350 ft-c provided by 40-w Ken-Rad "Cool White" fluorescent bulbs, and a 24-hr cycle of 12-hr light and 12-hr dark. These conditions will be referred to as "standard conditions" in all future sections.

Because *Stigeoclonium* and *Caespitella* are sessile forms, it was necessary to devise special methods to observe the erect and basal systems with equal facility. The following method proved to be the most satisfactory of the several tried. Erlenmeyer flasks (125 ml) were filled with 100 ml of BBMPTB$_{12}$ and a piece of glass (½ × 3 in.) was placed in each flask. The flasks were autoclaved and subsequently inoculated with a tuft of algal filaments. After an appropriate period, the glass was removed and the algae growing on the glass in the region from ¼–1 in. below the upper surface of the culture medium were examined with a microscope. This arbitrary limit was chosen because the more luxuriant growth usually occurred near the surface of the culture medium, especially in unaerated cultures.

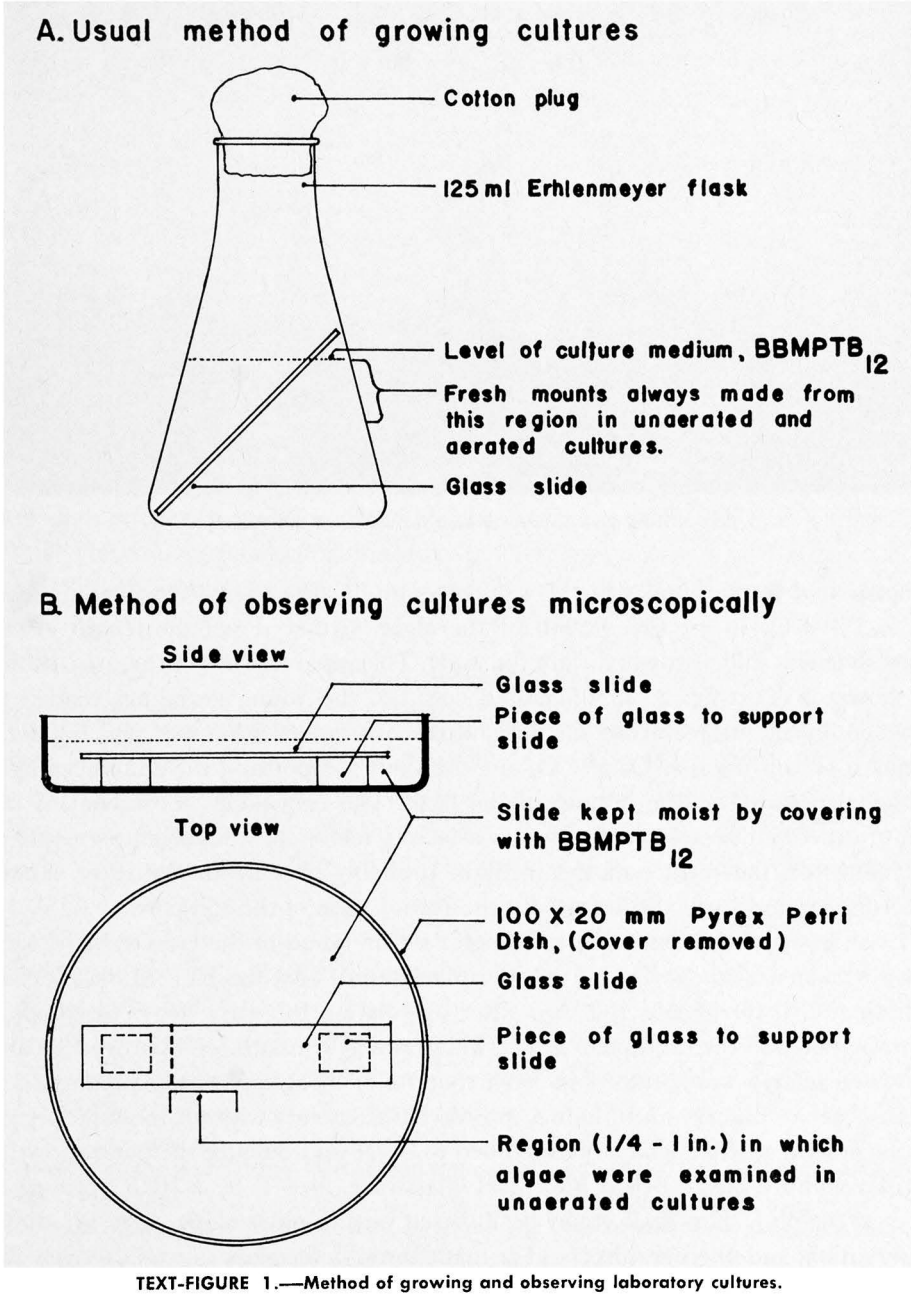
[6] Calbiochem, Los Angeles, California. Tris-(Hydroxymethyl) Aminomethane will be referred to as TRIS in the following sections.

[7] None of the isolates required Vitamin B_{12} for growth. However, because the growth of several of the isolates was enhanced by Vitamin B_{12}, and none was inhibited by it, B_{12} was added routinely to the culture medium.

[8] The minor element solutions for BBMP are outlined on pp. 10–11.

The glass slides were examined with a Bausch and Lomb stereoscopic binocular microscope and with a 50 × Leitz water-immersion objective. The method of growing the algae and making observations with the microscope is shown in Text-fig. 1.

Aeration of the cultures greatly enhanced the amount of growth of all of the



TEXT-FIGURE 1.—Method of growing and observing laboratory cultures.

TEXT-FIGURE 2.—Apparatus used for the aeration of cultures

isolates used in this study (Fig. 1–8); therefore, in flasks aerated with a mixture of 2–5% CO_2 in air the growth of the algae farther down the length of the glass slide was sufficiently luxuriant for study. The apparatus used to aerate cultures is shown in Text-fig. 2. In unaerated cultures, the young germlings tended to develop on the surface of the medium, often covering it with a mat which greatly reduced the diffusion of O_2 or CO_2 and even more important, the amount of light which penetrated to the bottom of the flask. The continuous movement of the culture medium in aerated flasks was clearly a factor in reducing the growth of germlings on the surface of the medium probably because the latter no longer afforded an undisturbed substrate for the germination of the zoospores.

Even in aerated flasks, fresh mounts for examination of the erect systems were always taken near the surface of the culture medium (Text-fig. 1A). Morphological examinations were made 1–2 days after inoculation for early stages in zoospore germination and then routinely at 1–2 weeks and at 1 month. Zoosporogenesis and zoospore release were studied in fresh mounts or in hanging-drop preparations.

In order to observe and photograph the basal systems without the interference of the erect filaments, which often tended to cover and obscure the basal growths, a tissue culture flask (Bellco Biological Glassware, Size T–9, #1015) proved to be very helpful. The flask could be inverted on the microscope stage for direct observation, and photographs could be made through the glass.

Measurement of cell size was made either directly with a stage micrometer or by comparing photographs of the organism and of the stage micrometer at the same magnification and degree of enlargement.

Several of the isolates were transplanted to a natural habitat in order to correlate laboratory observations with the growth of the organism in nature. The following procedure was used. Glass slides (1 × 3 in.) were etched with a diamond pencil (both code number of the organism, and cross-hatching to roughen the surface area) and placed in the bottom of a Pyrex Petri dish (100 × 20 mm).[9] The slides were then covered with $BBMPTB_{12}$, and the dish subsequently inoculated with a tuft of the algal filaments. After zoospores had been released, two of the slides covered with young germlings[10] were secured to a wire rack within a wire-enclosed wooden frame box (Fig. 9, 10). In every case an etched, blank, control slide was placed between the two "*Stigeoclonium* slides" to insure the accuracy of the observations, and to prevent confusion resulting from colonization by contaminants. The box was submerged in the Blanco River at San Marcos, Texas (Fig. 11–13), and allowed to remain for 2 weeks, after which the slides were removed and examined.

Colony characteristics were studied in 60 × 13 mm Pyrex Petri dishes of $BBMPTB_{12}$ solidified with 15 g/liter of Difco Bacto-Agar. Observations of colony characteristics were made at 1 month.

The photographic equipment used in this work included a Zeiss Super Contaflex, single-lens reflex camera with adaptable close-up lenses; a Kodak Pony camera on a Wild Heerbrugg stereoscopic binocular microscope; a Zeiss Icon Camera and a Zeiss Winkel photometer attachment on a Bausch and Lomb monocular microscope.

Taxonomic History

In the first monograph dealing with the world-wide distribution of the genus *Stigeoclonium* since the classical work of Kützing (1843, 1845, 1849, 1853), Islam (1963) has listed, in great detail, the major taxonomic works concerning *Stigeoclonium* sp. from Europe, North America, South and Central America, Asia, Africa, Australia, and New Zealand. He also has presented a chart showing the geographical distribution of approximately 100 described species and varieties of *Stigeoclonium,* although he states that "this species list does not necessarily correspond to the species accepted in this work." No effort will be made by the writers to repeat these lists; however, some of the information of a general nature will be reviewed here, from a slightly different point of view.

Kützing established the genus *Stigeoclonium* (originally spelled *Stygeoclonium*) in 1843 with the transfer of two organisms from the genus *Conferva* and three from

[9] Sometimes the etched slides were placed in an upright position around the sides of a 250–ml beaker.

[10] The germlings were 5–7 days old. Two germling-covered slides and one blank control slide were used for each organism.

Draparnaldia. Kützing's original delimitation of the genus (1843, p. 253) is as follows:

Trichoma tenerrimum, ramosum, ramulis simplicibus subulatis obseccum. *Cellulae gelineae* tenuissimae, abbreviate; *amylideae* in fasciam transversalem collapsae, tandem in *opseospermata* quaternata, turgida transeuntes.

Trichomes very delicate, branched, ending in simple awl-shaped branches. Cells gelatinous, very narrow, short; cell contents dividing transversely, finally becoming four turgid sperm-like parts.

The five species of *Stigeoclonium* established by Kützing in 1843 were: *S. stellare* (Ag.) Kütz., *S. uniforme* (Ag.) Kütz., *S. subsecundum* (Kütz.) Kütz., *S. biasolettianum* (Kütz.) Kütz., and *S. tenue* (Ag.) Kütz. In 1845 Kützing transferred six more species previously assigned to the genera *Chaetophora, Conferva,* or *Draparnaldia* by Dillwyn (1802), Agardh (1824), Hassall (1845) or Kützing himself to the genus *Stigeoclonium*. He erected the species *S. amoenum* Kütz. and *S. crassiusculum* Kütz. He also rearranged and divided some of his original species, placing *S. uniforme* (Ag.) Kütz. under *S. tenue* (Ag.) Kütz. as *S. tenue* var. *uniforme,* and separating *S. stellare* Kütz. into two species, namely *S. stellare* Kütz. and *S. irregulare* Kütz. *Stigeoclonium stellare* and *S. irregulare* were both later considered to be growth forms of *S. tenue* (Ag.) Kütz.; therefore, according to the International Code of Botanical Nomenclature in 1956, *Stigeoclonium tenue* (Ag.) Kütz. is considered to be the lectotype of the genus. These changes are graphically presented in Table 3.

The specific descriptions given by Kützing (1843, 1845) were brief and inadequate, seldom more than three or four lines in length, and unillustrated. Specific distinctions were, for the most part, based on the habit of the plant, color, presence or absence of hairs, cell width and length, and degree of branching.

In his classical work "Species Algarum" Kützing (1849) described 24 species of *Stigeoclonium,* together with several varieties. In "Tabulae Phycologicae" (1853), he raised three varieties to specific rank, created one new species, and transferred *Draparnaldia nudiuscula* Kütz. to the genus *Stigeoclonium,* as *S. nudiuscula* Kütz. He illustrated most of these species with beautifully executed line drawings which, however, stressed only the erect portion of the plant and again emphasized such vegetative characters as cell width, cell length, branching, and hairs. These vegetative characters are always more or less variable, and Hazen (1902) pointed out that this fact has led to two taxonomic approaches "either of which leads to confusion." Kützing tended to treat all variations that he found as separate species. This position was supported by Hazen (1902), who credited some of the confusion in the literature to the fact that specimens had been *forced* to fit a given species description. After Kützing came a series of workers, namely, Rabenhorst, Hansgirg, Kirchner, and DeToni who tended to reduce previously described species to varietal rank. These reductions were often made without understanding the original

descriptions and, more often than not, they confused rather than clarified the situation.

The first American work on the genus *Stigeoclonium* was that of Francis Wolle (1887). Wolle's treatment (sec. Hazen, 1902) was based on the classical works of Rabenhorst and Kirchner. Hazen (1902) noted that Wolle in "Fresh Water Algae of the United States," listed some of Kirchner's species, and that these same species were later listed by DeToni (1889), the great compiler, as American species. Hazen's work (1902) was, perhaps, the first important American contribution. Hazen used the generic name *Myxonema* Fries instead of *Stigeoclonium* Kützing. The question of *Myxonema* vs. *Stigeoclonium* was reviewed by Nordstedt (1906) and Islam (1963), and need not be discussed again here. The International Botanical Congress of 1910 kept *Stigeoclonium* Kützing (1843) as the *nomen conservandum* over the earlier name *Myxonema* Fries (1825).

Heering (1914) compiled a list of the European species of *Stigeoclonium*. Collins (1928) followed Hazen (1902) with a list of the United States species, adding two new species of his own. In 1963, after a careful study of European and American (excluding Hazen) exsiccatae, Islam combined and regrouped the previously described species of *Stigeoclonium,* ending with 28 species. Of these 28, he considered 10 to be "good species," 13 "possibly good species" and 5 "doubtful species." The species of *Stigeoclonium* recognized by Islam (1963) are listed in Table 2. It is interesting to note that 9 of the 10 species which Islam considered to be "good species" were also listed by Kützing in 1853.

A chronological chart of the major authors who have treated the genus *Stigeoclonium* from the time of Kützing (1843) to Islam (1963)[11] immediately reveals the "name-switching" that has occurred within the genus; that is, the combining of species, the separating of species, the elevation of varieties to specific rank, the reduction of species to varieties, etc. Table 3 presents these changes in graphic manner. The name of a given species should be followed across the table from 1843 to 1963. Names on the same level show that no change has been made in the taxonomic treatment of the organism. The dotted lines indicate changes made by later workers in the treatment of earlier workers.

These many changes indicate the uncertainty that exists with regard to species identification in *Stigeoclonium*. It is probable that a combination of several of the following factors account for this confusion: (1) the descriptions of the original species were incomplete and inadequate; (2) the original descriptions have been misinterpreted and misunderstood by later authors; (3) the vegetative characters (cell width, cell length, presence or absence of terminal hairs, color, degree of branching) used in the first descriptions are themselves variable; (4) two different plants growing close together have been identified as the same species; (5) different

[11] The work of Printz (1964) was not included in the chart. Printz merely listed previously described species and did not evaluate the validity of such species.

TABLE 2. *The species of Stigeoclonium recognized by Islam, 1963*

"Good Species"	*"Possibly Good Species"*
1. *S. amoenum* Kütz.	1. *S. aestivale* (Hazen) Collins
2. *S. fasciculare* Kütz.	2. *S. biasolettianum* Kütz.
3. *S. flagelliferum* Kütz.	3. *S. carolinianum* Islam
4. *S. longipilum* Kütz.	4. *S. elongatum* (Hass.) Kütz.
5. *S. lubricum* (Dillw.) Kütz.	5. *S. farctum* Berthold
6. *S. pachydermum* Prescott	6. *S. lebelii* Islam
7. *S. protensum* (Dillw.) Kütz.	7. *S. nanum* (Dillw.) Kütz.
8. *S. subsecundum* Kütz.	8. *S. nudiusculum* Kütz.
9. *S. subuligerum* Kütz.	9. *S. paihiae* Islam
10. *S. tenue* (Ag.) Kütz.	10. *S. segarae* Islam
	11. *S. stagnatile* (Hazen) Collins
	12. *S. thermale* A. Br.
	13. *S. variabile* Nägeli

"Doubtful Species"

1. *S. curvirostrum* Skuja
2. *S. helveticum* Vischer
3. *S. nelsonii* Prescott
4. *S. pusillum* (Lgb.) Kütz.
5. *S. setigerum* Kütz.

stages in the life cycle of the same species of *Stigeoclonium* have been identified as different species.

As has been stated previously, Islam's work (1963) was based almost entirely on the examination of herbarium materials. Although he mentioned that some culture work was done, he did not tell which organisms were cultured, what medium was used, or the conditions of culture. One gets the impression that three or four of the isolates available from the Culture Collection of Algae, Indiana University, were studied briefly. Although Hazen (1902) did attempt to grow a few plants in the natural habitat, most of his observations were based on collections in the northeastern part of the United States. None of the other workers concerned with major taxonomic revision of the genus *Stigeoclonium* has, to the writers' knowledge, done any culture work at all.

Although Islam's monograph represents a careful and comprehensive treatment of the genus *Stigeoclonium* from the existing herbarium specimens, it illustrates, in the opinion of the writers, the futility of basing the circumscription of such algal species on herbarium studies alone. Examination of Islam's key and descriptions of species leaves considerable uncertainty as to the precise definition of each of the several species. There is much confusion and uncertainty generated in the use of such terms as "growth form," "early stage," or "young form" in discussing

TABLE 3. A GRAPHIC COMPARISON OF STIGEOCLONIUM SPECIES FROM 1843 TO 1963

A.K.M. NURUL ISLAM 1963	F. S. COLLINS 1928	W. HEERING 1914	T.E. HAZEN 1902	J. BAPTISTE DE TONI 1889	F. WOLLE 1887	F.T. KÜTZING 1853	F.T. KÜTZING 1849	F.T. KÜTZING 1845	F.T. KÜTZING 1843
S. subsecundum (Kütz.) Kutz. S. subsecundum (Kütz.) Kütz. var. subsecundum S. subsecundum var. tenuis Nordt. (Emend.)	S. subsecundum Kütz.	S. subsecundum Kütz.	Myxonema subsecundum Kütz.	S. subsecundum Kütz. S. subsecundum var. tenulus Nordst.	S. subsecundum Kütz.	S. subsecundum Kütz.	S. subsecundum Kütz.		S. subsecundum Kütz. (= Conferva subsecunda Kütz.)
S. Biasolettianum Kütz.				S. Biasolettianum Kütz.		S. Biasolettianum Kütz.	S. Biasolettianum Kütz.		S. Biasolettianum Kütz. (= Drap. Biasolettiana Kütz.)
S. variabile Naeg. (Emend.)		S. variabile Naegeli		S. variabile Naegeli S. variabile var. minus Hansg.		S. variabile Naegeli	S. variabile Naegeli		
						S. subspinosum Kütz.	S. subspinosum Kütz. S. subspinosum var. falklandicum Kütz. (= Draparnaldia pusilla Hook. et Harr.)		
	S. falklandicum Kütz.			S. falklandicum Kütz. S. falklandicum var. longearticulatum Hansg.		S. falklandicum Kütz.			
S. elongatum (Hassall) Kütz.						S. elongatum Kütz.	S. elongatum Kütz. (= Drap. elongata Hassall)		
	S. attenuatum (Hazen) comb. nov.		Myxonema attenuatum sp. nov.						
	S. longearticulatum (Hansg.) Heering	S. longearticulatum (Hansg.) Heering							
S. thermale A. Braun.	S. thermale A. Braun.	S. thermale A. Braun.	Myxonema thermale A. Braun. (= Drap. uniformis Ag.)	S. thermale A. Braun.	S. thermale A. Braun.	S. thermale A. Braun.	S. thermale A. Braun.		
		S. pygmaeum Hansgirg							
		S. polymorphum (Franke) Heering							
	S. subsimplex nov. sp.								
S. tenue (Ag.) Kütz.	S. tenue (Ag) Kütz.	S. tenue Kütz.	Myxonema tenue (Ag) Rabenh.	S. tenue (Ag.) Rabenh.	S. tenue Kütz.	S. tenue Kütz.	S. tenue Kütz.	S. tenue Kütz.	S. tenue Kütz. (= Drap. tenuis Ag.) (= Conferva exigua Dillw.)
S. tenue (Ag.) Kütz. var. tenue		S. tenue Klebsi S. tenue Pascheri S. tenue Westi		S. tenue var. genuinum (Kütz.) Kirch. S. tenue var. lubricum (Dillw.) Rabenh. S. tenue var. irregulare (Kütz.) Rabenh. S. tenue var. uniforme (Ag.) Kütz. S. tenue var. bulbiferum Wolle S. tenue var. gracile Kütz. S. tenue var. epiphyticum Hansg. S. tenue var. lyngbyaecolum Hansg. S. tenue var. minus Hansg.	S. tenue var. genuinum Kirch. S. tenue var. lubricum Rabenh. S. tenue var. irregulare Rabenh. S. tenue var. uniforme Rabenh. S. tenue var. bulbiforme Wolle				
S. tenue (Ag.) var. uniforme (Ag.) Kütz.							S. tenue var. uniforme	S. tenue var. uniforme	
							S. tenue var. gracile		
				S. debile Kütz.		S. uniforme Kütz.			S. uniforme Kütz. (= Drap. uniformis Ag.)
						S. debile sp. nov. (Kütz.)			
						S. gracile Kütz.			
						S. stellare Kütz.	S. stellare Kütz.	S. stellare Kütz.	S. stellare Kütz. (= Conferva stellaris Roth) (= Conferva stellaris Ag.)
						S. irregulare Kütz.	S. irregulare Kütz. S. irregulare var. β natans Kütz.	S. irregulare Kütz.	
S. lubricum (Dillw.) Kütz.	S. lubricum (Dillw.) Kütz. S. lubricum (Dillw.) Kütz. var. varians (Hazen) comb. nov.	S. lubricum Kütz. S. lubricum Kütz. var varians Hazen	Myxonema lubricum (Dillw.) Fries Myxonema lubricum (Dillw.) Fries var. varians var. nov.			S. lubricum Kütz.	S. lubricum Kütz.	S. lubricum Kütz. (= Conferva lubrica Dillw.)	
S. subuligerum Kütz.	S. subuligerum Kütz.	S. subuligerum Kütz. (includes S. subspinosum Kütz.)	Myxonema subuligerum Kütz.			S. subuligerum Kütz.	S. subuligerum Kütz		
S. protensum (Dillw.) Kütz.		S. protensum Kütz.	S. protensum (Dillw.) Kütz.	S. protensum (Dillw.) Kütz.	S. protensum (Dillw.) Kütz.	S. protensum Kütz.	S. protensum Kütz.	S. protensum Kütz. (= Conferva protensa Dillw.)	
				S. protensum var. subspinosum (Kütz.) Rabenh.					
				S. protensum var. subuligerum (Kütz.) Rabenh.					
							S. condensatum Kütz. (= Drap. condensata Hassall)		
S. nanum (Dillw.) Kütz.	S. nanum (Dillw.) Kütz. S. nanum forma subsimplex Collins	S. nanum Kütz.	Myxonema nanum (Dillw.) Kütz.	S. nanum (Dillw.) Kütz.	S. nanum (Dillw.) Kütz.		S. nanum Kütz. (= Conferva nana Dillw.)		
S. setigerum Kütz.		S. setigerum Kütz.		S. setigerum Kütz.		S. setigerum Kütz.	S. setigerum Kütz.	S. setigerum Kütz. (= Conferva oscillatoria Kütz.) (= Gloeotila oscillaria Kütz.)	
S. amoenum Kütz. S. amoenum Kütz. var. amoenum Kütz.	S. amoenum Kütz. S. amoenum forma biforme nova forma	S. amoenum Kütz.	Myxonema amoenum Kütz.	S. amoenum Kütz. S. amoenum Kütz. var. novizelandicum Nordst.	S. amoenum Kütz.	S. amoenum Kütz.	S. amoenum Kütz. S. amoenum Kütz. var. pulchellum	S. amoenum Kütz.	
S. amoenum Kütz. var. novizelandicum Nordst. S. amoenum Kütz. var. insigne (Naeg) comb. nov. S. amoenum Kütz. var. aucklandicum var. nov.									
	S. ventricosum (Hazen) comb. nov.		Myxonema ventricosum sp. nov.						
		S. insigne Naegeli							
S. longipilum Kütz. (Emend.)		S. longipilum Kütz.	S. longipilum Kütz.	S. longipilum Kütz.	S. longipilus Kütz.	S. longipilus Kütz.	S. longipilus Kütz.	S. longipilus Kütz. (= Chaetophora draparnaldioides Kütz.)	
				S. longipilum Kütz. var. minus Hansg.			S. longipilus Kütz. var. maius A Braun.		
	S. minus (Hansg) comb. nov.		S. radians Kütz.	S. radians Kütz.	S. radians Kütz.	S. radians Kütz.	S. radians Kütz.		
			S. fastigiatum (Ralfs) Kütz.	S. fastigiatum (Ralfs) Kütz.	S. fastigiatum (Ralfs) Kütz. (= Chaetophora fastigiatum Ralfs)	S. fastigiatum Kütz.	S. fastigiatum Kütz.		
S. fasciculare Kütz. (Emend.)		S. fasciculare Kütz.	S. fasciculare Kütz.	S. fasciculare Kütz.	S. fasciculare Kütz.	S. fasciculare Kütz.	S. fasciculare Kütz.		
S. fasciculare Kütz. var. fasciculare S. fasciculare var. glomeratum (Hazen) comb. nov.									
	S. glomeratum (Hazen) comb. nov.		Myxonema glomeratum sp. nov.						
S. pusillum (Lyngb.) Kütz.				S. pusillum (Lgb.) Kütz.		S. pusillum Kütz.	S. pusillum Kütz.		S. pusillum Kütz. (= Conferva pusilla Lgb.)
				S. pusillum (Lgb) Kütz. var. irregulare Rabenh.					
S. flagelliferum Kütz.	S. flagelliferum Kütz.	S. flagelliferum Kütz.	Myxonema flagelliferum (Kütz.) Rabenh.	S. flagelliferum Kütz.	S. flagelliferum Kütz.	S. flagelliferum Kütz.	S. flagelliferum Kütz.		S. flagelliferum Kütz. (= Drap. tenuis var. elongata Ag.)
				S. flagelliferum Kütz. var. crassiusculum (Kütz.) Rabenh.	S. flagelliferum Kütz. var. crassiusculum Kütz.				
						S. crassiusculum Kütz.	S. crassiusculum Kütz.		S. crassiusculum Kütz.
				S. plumosum Kütz.		S. plumosum Kütz.	S. plumosum Kütz.		
S. nudiusculum (Kütz) Kütz. (Emend.)		S. nudiusculum Kütz.	S. nudiusculum Kütz.	S. nudiusculum Kütz.	S. nudiusculum Kütz.	S. nudiuscula Kütz. (= Drap. nudiuscula Kütz.)	Drap. nudiuscula Kütz.		Drap. nudiusculum Kütz.
				S. rangoonicum Zell.					
S. farctum Berthold		S. farctum Berthold		S. farctum Berthold					
		S. Huberi Heering							
S. aestivale (Hazen) Collins	S. aestivale (Hazen) comb. nov.		Myxonema aestivale sp. nov.						
	S. autumnale nov. sp.								
S. stagnatile (Hazen) Collins	S. stagnatile (Hazen) comb. nov.		Myxonema stagnatile sp. nov.						
		S. chroolepiforme (Szym.) Heering							
S. Lebelii sp. nov.									
S. curvirostrum SKuja									
S. carolinianum sp. nov.									
S. helveticum Vischer									
S. segarae sp. nov.									
S. Nelsonii Prescott									
S. pachydermum Prescott S. pachydermum Prescott var. pachydermum S. pachydermum var. Whitfordii var. nov. S. pachydermum var. Prescottii var. nov.									
S. paihiae sp. nov.									

the species. The following quotations from Islam's monograph (1963, pp. 82, 86) illustrate this point:

It has been noticed that the reduced or growth-form of *S. lubricum* and *S. flagelliferum* may appear as *S. fasciculare*. Similarly, *S. nudiusculum* and *S. Nelsonii* seem very close to it.

Sometimes the young stages of *S. subsecundum* may look like *S. aestivale*.

One might also note a statement by Forest (1954, p. 87) in reference to the identification of *Stigeoclonium nanum* (Dillw.) Kütz.:

This species was recorded in 3 or 4 cases, and, if the species had been preserved immediately, the species could have been written with some assurance. Since some of them were allowed to grow in the laboratory, however, they developed similarity to other species—*S. attenuatum, S. stagnatile,* and *S. lubricum.*

The writers are well aware of the debate that has existed for many years with regard to the validity of taxonomic work based on cultural studies of algae. The following quotations clearly and succinctly define the positions in the opposing phycological armies.

Dr. G. W. Prescott (1964, p. 23–24) says:

. . . [S]ome (not all) culturing of algae has introduced taxonomic confusion when characterization of species has been attempted only from material under artificial conditions. We all know, of course, that algae in culture do not maintain their natural or original morphology and that they do not pass through normal life history stages. Also they vary in their particular vegetative or asexual reproductive techniques in cultivation, according to the type of medium used and according to variables in other ecological factors. Hence it is not easy to characterize algae under artificial conditions and in many instances it is not feasible, for these expressions on one medium in one laboratory seldom are reproducible in others.

The splitting or lumping of species based on culture studies is, in my opinion, very risky. Some students are disposed to throw two species together when it is shown that variable, and what might be called "abnormal," expressions of both are similar, if not identical. We should expect two species descended from the same parent to have at least some features in common. It is not surprising that variations from the normal would appear similar when placed in artificial culture.

However, van den Hoek (1964, p. 54) expresses a somewhat different point of view:

As a general conclusion I should like to express my opinion that investigations of cultures grown under controlled conditions combined with observations on living material from nature and, if useful, on herbarium collections should be the base for taxonomic studies, not only of unicellular and colonial forms, but of many pluricellular forms as well. In many pluricellular groups such as the genera *Stigeoclonium, Chaetophora, Draparnaldia, Draparnaldiopsis,* and *Cloniophora,* we may expect a consider-

able morphological plasticity and possibly large overlaps of morphological characteristics between the species. . . . For this reason such generic revisions as those of *Draparnaldia* by Forest (1956) and *Cloniophora* by Islam (1961), which are mainly based on preserved material, leave a feeling of uncertainty (evidently experienced by Forest), since the validity of the criteria employed in them remains problematic.

There have been several efforts in the past to study and grow *Stigeoclonium* plants in nature and in the laboratory. However, the present investigation represents the first effort to bring a number of *Stigeoclonium* isolates into bacteria-free culture and to compare the morphology of the organisms in a defined culture medium under controlled environmental conditions with that of the same plants "transplanted" to the natural environment from which many of them were isolated.

The history of the genus *Caespitella* Vischer is neither as lengthy nor as colorful as that of *Stigeoclonium*. The genus *Caespitella* was established by Vischer (1933) to encompass some organisms that he first thought were members of the genus *Stigeoclonium*. However, he noted several differences,[12] and on this basis established a new genus. A footnote by Fritsch (1935) and a brief note by Forest (1956)[13] constitute the only subsequent mention of the genus by any later workers. The disposition of the genus by the writers will be discussed later in this report.

The Validity of Taxonomic Criteria Currently Used to Circumscribe Species in the Genus *Stigeoclonium*

STATEMENT OF THE PROBLEM—Islam (1963) brought together much of the literature concerning the life history, reproduction, and general vegetative morphology of plants associated with the genus *Stigeoclonium* Kützing.[14] A knowledge of the efforts of previous workers with this difficult group of filamentous algae leads one inescapably to the conclusion that it is dangerous, and indeed futile, to generalize as to the behavior of any particular species, or group of species, from the accounts given of these species in the literature. Various workers expressed doubt as to their own identifications, and no certain identification can be made by any present worker from their descriptions.

As has been mentioned, the vegetative morphology of *Stigeoclonium* has been studied largely from preserved specimens collected at different times and in different places. Such a study, based on herbarium specimens and largely employing the

[12] Most important was apical growth. *Stigeoclonium* has intercalary growth (erect filaments only). Refer to pp. 37, 73 of this paper.

[13] Forest (1956) mentions the genus *Caespitella* briefly in a treatment of the evolution of the filamentous Chlorophyceae. He does not, however, comment on the validity of the genus.

[14] The genus *Caespitella* Vischer was not mentioned by Islam (1963). Fritsch (1935) said that the evidence for the occurrence of *Caespitella* is not entirely convincing.

type method,[15] in such a genus as *Stigeoclonium* presents, in the opinion of the writers, several inherent difficulties, as follows:

1. It is not possible to correlate observations. Contrary to Islam's statement (1963, p. 45), "Geographical variation is not so pronounced in aquatic plants as it is in higher land plants," the writers believe that the chemical constituents of the stream or pond, the temperature of the water, the amount of light, as well as other environmental factors, exert as great an influence on the morphology of plants such as *Stigeoclonium* as has been shown to be the case in some of the land plants. The effect of these factors is difficult to determine and impossible to control in the natural environment.

2. One can never be sure of the age, or state of maturity, of the specimen examined in a herbarium collection. Therefore, a comparative study of specimens from several places may cause one to assume relationship or identity between similar, but unrelated, forms.

3. An investigator is often forced to rely on poorly preserved materials and, perhaps, to place undue weight on the opinion of the collector as to the identity of the species.

However, in spite of the above-mentioned pitfalls, the opinion of many previous investigators has been that growth "in nature" is "normal"; whereas, growth "in culture" results in "abnormal" or "atypical" morphological expressions.[16] Peirce and Randolph (1905) stated that some of the responses of organisms in "pure culture" may, in fact, be unnatural because of artificial media, confinement in the culture vessel, or the freedom of the algae from competition with other organisms and their products. Islam (1963, p. 18) said: "It is obvious that in different media these plants will grow differently. . . ." and,

> . . . [T]hese filamentous plants are extremely sensitive and variable in culture media where they are not subjected to all physical and chemical conditions of natural habitat.

Surprisingly enough, in view of the tendency to dismiss cultural studies as "atypical," very few investigations in defined inorganic media under controlled conditions have been performed. Nägeli (1855), Berthold (1878), Gay (1891), Tilden (1896), Fritsch (1903), Pascher (1905, 1906a, b, c), and to some extent even Vischer (1933), did not specify the conditions of culture they employed. Reynolds (1951) and Butcher (1931, 1932, 1946, 1950) studied the growth of species of *Stigeoclonium* which colonized glass slides suspended in ponds or streams. Both expressed doubt as to the identity of the organisms thus collected. Reynolds

[15] That is, comparing all subsequent forms with a given form, the type, in which all the attributes of the species are assumed to be visible.

[16] As often used in the literature, the term "in culture" seems to imply that only one set of conditions could be brought about. However, the writers emphasize that the investigator may produce any desired conditions "in culture." Some cultural conditions could, indeed, produce "abnormal" growth, but it does not follow that all cultural conditions would do so.

(1951) said that such organisms thus collected could be identified only if they were grown in culture so that all the stages of development could be seen. Godward (1942), Reynolds (1951), and Change (1952) cultured species of *Stigeoclonium* in inorganic media,[17] and reported that the morphology was not unlike that found in nature.[18] A completely inorganic medium was used by Klebs (1896) and Uspenskaia (1936a, b). Klebs (1896) succinctly stated the problem of species determination in *Stigeoclonium*. He said that the characters used to delimit species were the most variable in the genus, and that the extent of their variability was not known in any species. Despite the efforts of several recent investigators, the fact remains that identification of *Stigeoclonium* species is still impossible in some cases, and doubtful, or uncertain in others.

The writers agree with Islam (1963, p. 18) that a cultural study should endeavor to determine "maximum vegetative expressions of the thallus" and not to "merely show variations." However, if morphological variations do exist in organisms grown in a carefully constituted culture medium,[19] these variations must be interpreted as normal phenotypic expressions of the organism and the concept of the "species" expanded to encompass them. In addition, if the classical characters of the erect vegetative thallus are found to be inconsistent, unreliable and overlapping, less emphasis must be placed on these characters and other more reliable criteria formed to delimit species.

The phenotype of any organism is determined by the genome of the organism and the extent to which the environment enhances or suppresses these genetic potentialities. Some range of morphological variation is, therefore, inherent in any organism. Until the sexual process of *Stigeoclonium* can be evoked and controlled in the laboratory and the genetic integrity of questionable organisms definitely established, the criteria for circumscribing a "species" depend largely on the judgment of the investigator as to those morphological and physiological characters which are most reliably and consistently expressed.

The writers concur with the following statement made by Islam (1963, p. 44):

> It is assumed that in a particular habitat two fully-grown but different kinds of plants may exist side by side and in that event they should be regarded as separate species, or in other words, *a particular species at a particular time at a particular place will look uniform and will not show extreme variations except young-adult growth-forms.*[20]

After a careful study of 20 *Stigeoclonium* and *Caespitella*-like isolates, it is our opinion that: (1) the genus *Stigeoclonium* probably contains fewer distinct entities

[17] Enriched with soil supernatant, however.

[18] Reynolds and Godward employed adaptations of the various media developed by Chu (1942).

[19] In which the growth of several of the organisms can be demonstrated to approximate the growth of the same organisms in the waters of their natural environment.

[20] Italics added by the writers.

than has been previously supposed (cf. Forest, 1955); (2) the use of the morphological characteristics of the erect filaments for specific determination has caused much of the taxonomic confusion that exists today; and (3) the vegetative morphology of the basal system, largely ignored in the past, is a more reliable taxonomic criterion than is the erect system.

The discussion of the taxonomic criteria usually associated with *Stigeoclonium* and the descriptions of the isolates employed in this investigation provide evidence for the above statements.

HETEROTRICHY—Heterotrichy has been defined by Fritsch (1942) as the "successive development of the plant body in two planes." He used this character to distinguish the order Chaetophorales from the order Ulotrichales, in that the vegetative thallus of the former is usually differentiated into an erect system of branching filaments and a prostrate system which attaches the plant to the substratum.[21]

The relative degree of development of the erect system and the prostrate system differs significantly in some species of *Stigeoclonium*. Fritsch (1935) noted that the development of the erect system is usually "in inverse ratio" to the development of the prostrate system. In addition, the proportion of development of the two systems in one species is subject to environmental variation. Consequently, attributes based on macroscopic appearance (such as habit of the plant or size of the thallus) tend to overlap or differ according to environmental conditions[22] and are not conclusive for species identification.

Under the conditions maintained in this study sufficient nutrients for optimum growth were available, and the erect filaments were not forcibly detached from the system by contact with any outside agent. Consequently, the erect systems in most cultures attained a greater length than corresponding cultures in nature. However, in *S. farctum* (isolates 19–5–V, 5–3C, 5–3F), which has an elaborate disc-like basal system (Fig. 227), the erect filaments never attained the length of those of *S. aestivale* (isolates 8–3, HP 4, Var 5), in which the basal system is a small branching filament of restricted growth (Fig. 50).

An increase in the amount of erect growth, accompanied by complete development of the basal system, resulted when the cultures were aerated with a mixture of 2–5% CO_2 in air (Fig. 1–8). A medium with a lower pH (BBMP—pH 6.3) than the control medium (BBMPTB$_{12}$—pH 7.6) promoted extensive erect development, but sometimes inhibited the complete formation of the basal system. This was very apparent in *S. farctum* in which a smaller, less disc-like basal system

[21] Fritsch (1935) used the following system of classification: Order Ulotrichales; Family Ulotrichaceae and Order Chaetophorales; Families: Chaetophoraceae; Trentepohliaceae; Coleochaetaceae; Chaetosphaeridaceae.

Smith (1950) included the Ulotrichaceae, Chaetophoraceae, Trentepohliaceae, Coleochaetaceae, and Chaetosphaeridaceae as families of the single Order Ulotrichales.

[22] This does not necessarily imply *unfavorable* environmental conditions.

consistently formed. An organically enriched medium (3 parts BBMP: 1 part soil supernatant) encouraged complete development of the basal system, but suppressed growth of the erect filaments in some organisms. Again, in *S. farctum* the erect system was sometimes (Fig. 224, 225), but not always, reduced to a few short branches or to colorless multicellular hairs in such organically enriched medium. The basal system, however, remained a beautiful *Coleochaete*-like disc (Fig. 224). Plants of *S. farctum* growing on submerged slides in the Blanco River, San Marcos, Texas, frequently exhibited this same phenomenon. A similar, although much less frequent, reduction of the erect filaments of *S. farctum* was noted in plants growing near the interphase between the air and liquid in the control medium, $BBMPTB_{12}$. The basal system in this case was perfectly disc-like. On the basis of these observations, the writers take exception to the statement of Islam (1963, p. 37–38) to the effect that: "If the conditions are not 'optimum,' then a 'stunted' growth with more prostrate system usually develops." In these three instances, the "stunted" growth of the erect system of *S. farctum* can not be attributed entirely to the lack of "optimum" conditions. Even more important, it must be noted that the size of the basal systems in the organically enriched medium and in the river *equalled but did not exceed* the size of that in the control medium. Uspenskaia (1936b) found that increasing the concentration of nitrate in the culture medium caused a decrease in the size of the thallus of *S. tenue*. No mention of any corresponding increase or decrease in the development of the basal system was made.

Stigeoclonium species in which the basal system is poorly developed, or lacking entirely, usually produce rhizoids from the lower cells of the erect filaments to aid in attachment (Fig. 26). This rhizoidal development may be quite extensive (Fig. 78). Inasmuch as the writers agree with Gay (1891) that rhizoids are really modified branches, it is doubtful whether a species of *Stigeoclonium* in which attachment to the substratum is *entirely* by rhizoids should, in the strictest sense, be called heterotrichous.[23]

The heterotrichous nature of the *Caespitella*-like isolates (10–2, 18–3, and Ca 421) is often not immediately evident (Fig. 156). The ends of the spreading basal filaments become detached from the substratum, continue to proliferate, and thus obscure the "true" erect filaments which are composed of narrow, cylindrical cells. The "true" erect filaments are usually not very numerous.

ASEXUAL AND SEXUAL REPRODUCTION AND EARLY GERMLING STAGES—Reliable information about the process of reproduction, especially sexual reproduction, in species of the genus *Stigeoclonium* is almost completely lacking.[24] Accounts in the

[23] For this reason, the writers prefer to include *Stigeoclonium* in the Order Ulotrichales (Smith, 1950).

[24] With the possible exception of Godward's report (1942) of sexual reproduction in *S. amoenum*.

literature are confusing, often contradictory, and certainly in need of confirmation (especially Juller, 1937; Chang, 1952; and Singh, 1954). Because of questionable specific identification by previous workers, one should be cautious about generalizing regarding sexual reproduction of individual species or, indeed, of the genus as a whole; this has not been done by Chapman (1941, 1962), Fritsch (1935), or Smith (1950). Pascher (1906c) pointed out that different reproductive cells have been described for three morphologically similar *Stigeoclonium* isolates. He said this apparent difficulty in reconciling the reproductive cycle of similar organisms may lie in the different responses of the plant to the environment, or to the stage of growth of the plant. Until the reproductive process of any one *Stigeoclonium* isolate can be evoked and studied in the laboratory, and regrettably this is not now always possible, attempts to use attributes of the reproductive process (such as the shape of the zygote, Godward, 1942) in addition to other morphological characteristics in specific circumscription must be regarded as premature.

The diversity of the reproductive process in the genus *Stigeoclonium,* as reported by various investigators, can best be summarized in the form of a composite diagram with accompanying explanatory tables. Text-fig. 3 presents a schematic diagram of the most important reports of reproduction in *Stigeoclonium*—with the organisms grouped not according to species, but rather *according to similar reproductive behavior.* Such a synopsis makes the confused state of our present knowledge immediately apparent. Much of the confusion is probably the result of faulty or incomplete observations, incorrect interpretations, or mixed cultures.[25]

As can be seen in Text-fig. 3, there have been reports of reproductive cells produced by the erect portions of the thallus (THALLUS A), and other reports of reproductive cells produced by the so-called "*Palmella* stage"[26] (THALLUS B —really just a later stage of the erect thallus). With the exception of biflagellate gametes[27] (VII),[28] all of the cells, both asexual and sexual, derived *directly* from the erect thallus (THALLUS A) have been reported as quadriflagellate. Biflagellate gametes (IX, X), and biflagellate zoospores—some workers say gametes that develop parthenogenetically (VIII, XI, XII)—have been reported to originate from the "*Palmella* stage" (THALLUS B).

The zygote has been said to: (1) germinate directly (IX)—indicating gametic meiosis?; (2) form a small three- to four-celled prostrate thallus which subsequently releases quadriflagellate zoospores (X)—indicating an alternation of

[25] The writers have decided to exclude the contribution of Chang (1952) from Text-fig. 3. It is probable that she did not have a unialgal culture, but instead a mixture of *Stigeoclonium* and *Ulothrix.*

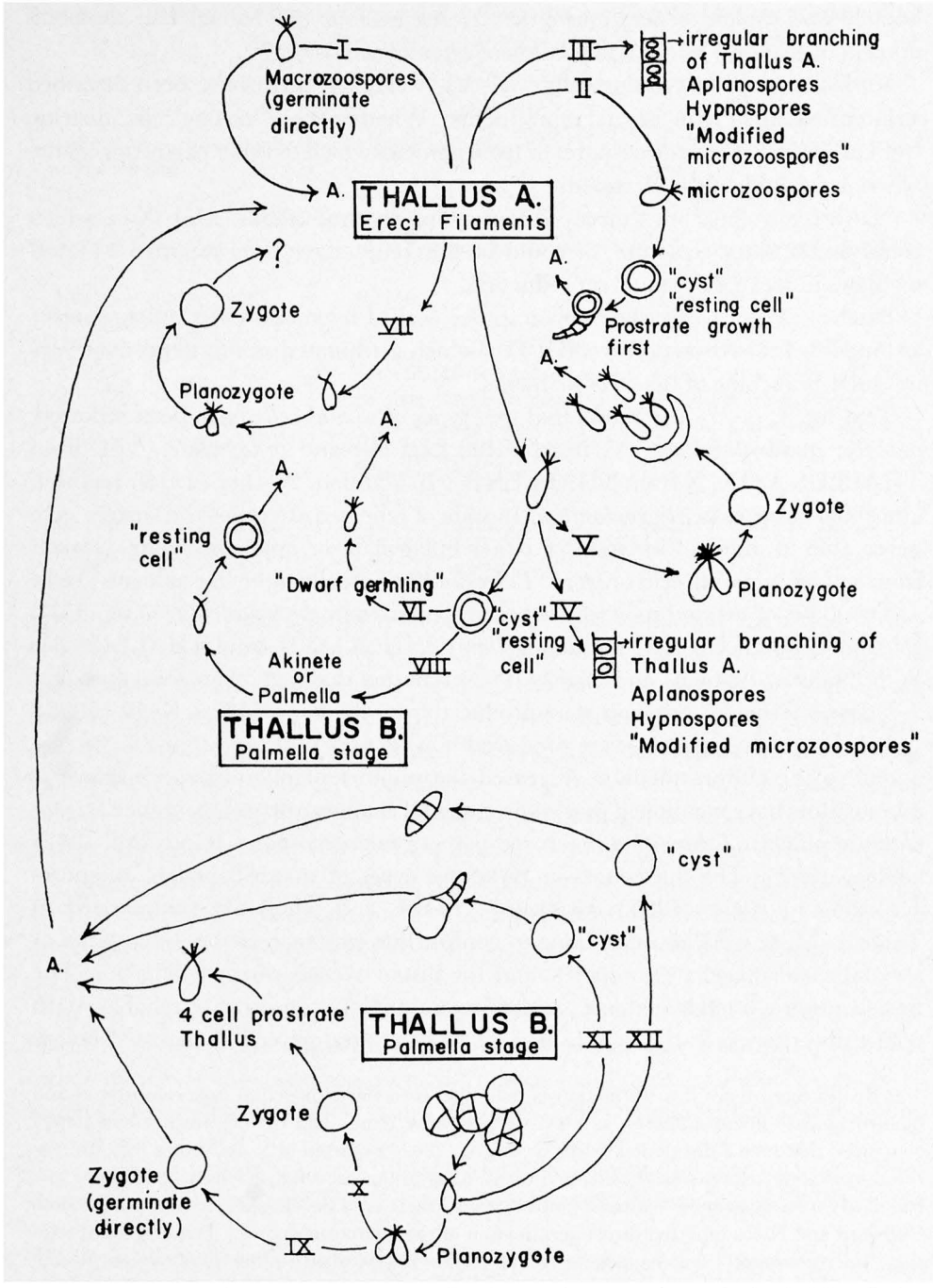
[26] The "*Palmella* stage" is discussed on p. 31.

[27] The reports of such biflagellate gametes from the erect filaments are not very convincing. Pascher (1906b, c) proposed that the biflagellate gametes of *Ulothrix* have been replaced by quadriflagellate gametes in *Stigeoclonium.*

[28] All of the Roman numerals refer to Text-fig. 3, p. 27, and to the explanatory chart accompanying Text-fig. 3, p. 26.

	Size, Length Width	No. per cell	Motile Period	Position Stigma	Cell formed . . .
I (Thallus A)	**Asexual quadriflagellate (Macrozoospores)**				
Pascher (1906a) *S. longipilum*	12-16μ 5-6μ	1-2	10-min.- 2 hrs.		
Pascher (1905, 1906b) *S. fasciculare*	12-16μ 8-9μ	1-2			May produce "Abnormal Macrozoospore Germling" which is 4-celled and produces Asexual Quadriflagellate Zoospores
Pascher (1906c) *S. nudiusculum*	17-20μ 9-11μ	1-2			New plant formed directly
Pascher (1906e) *S. tenue* ?	13-16μ 6-7μ				
Tilden (1896) *S. flagelliferum*	10-12μ 5-7.5 (10μ)	1-2-(16)	"Some time"		Prostrate part developed first.
Klebs (1896) *S. tenue* ?	12-14μ 5.5-6.5μ	1	3 hrs.		New plant directly as in *Ulothrix*.
Godward (1942) *S. amoenum*	8-10μ 5-9μ	1-several (Separated by fine walls)	"Short"		→ → → → rhizoid
Juller (1937) *S. subspinosum*	10-11μ 7-8μ	1	"Short"		→ → →
West (1904) *S. tenue*					
II (Thallus A)	**Asexual quadriflagellate (Microzoospores)**				
Juller (1937) *S. subspinosum*	8-9μ 6-7.5μ	2			Resting Cell → Prostrate Growth → Thallus A
Klebs (1896) *S. tenue* ?	7-9μ 5-6.3μ	4	24-48 hours		Green Resting Cell — Few days to several weeks → Prostrate Growth → Thallus A
Pascher (1906a) *S. longipilum*	9-12μ 3-5μ	2	Longer than Macro.		Resting Cell → ? → ?
III (Thallus A)	**Aplanospores from asexual quadriflagellate**				
Pascher (1906a) *S. longipilum*	9-12μ 3-5μ	2			
IV (Thallus A)	**Aplanospores from potentially sexual quadriflagellate**				
Pascher (1905, 1906b) *S. fasciculare*	9-12μ 5-6μ				
Pascher (1906c) *S. nudiusculum*	12-14μ 5-7μ				
V (Thallus A)	**Sexual quadriflagellate (Microzoospore)**				
Godward (1942) *S. amoenum*	7μ 5μ	Large number transverse divisions	12-24 hrs., cont. 1½ 2-3 days		Planozygote 10 min. - ½ hr. settle down → "star shaped" Orange Zygote → 4 ♂ 11μ long 9μ wide → Thallus A
Pascher (1905, 1906b) *S. fasciculare*	9-12μ 5-6μ				Planozygote 12 min. - 1½ hr. → Red Smooth Zygote → ?
Pascher (1906c) *S. tenue* ?	9-10μ 6μ				Planozygote → ?
Pascher (1906c) *S. nudiusculum*	12-14μ 5-7μ				? → Red Smooth Zygote → ?
VI (Thallus A)	**Asexual quadriflagellate**				
Pascher (1906c) *S. nudiusculum*	12-14μ 5-7μ				Red "Resting Cell" → "dwarf germling" 4 cells "zwergkeimlingen" → ♂ → Thallus A
Pascher (1906c) *S. tenue* ?	9-10μ 6μ				"Resting Cell" → ?
VII (Thallus A)	**Sexual biflagellate (Gametes)**				
West (1904) *S. tenue*					Planozygote → Smooth Zygote → ?
Pascher (1906a) *S. longipilum*		2			?
VIII (Thallus A and Thallus B)	**Asexual quadriflagellate and asexual biflagellate**				
Pascher (1905, 1906b) *S. fasciculare*	9-12μ 5-6μ				Resting Cell → Akinete Palmella stage → 4 ♂ 13μ long 7μ wide → ○ resting cell → Thallus A
IX (Thallus B)	**Sexual biflagellate (Gametes)**				
Tilden (1896) *S. flagelliferum* (microzoospores)	5-7μ 3.5-4μ	4-8			Planozygote → Smooth Zygote → Direct Germination → Thallus A
X (Thallus B)	**Sexual biflagellate (Gametes)**				
Juller (1937) *S. subspinosum*	8-9μ 7-8μ	4			Planozygote → Smooth Zygote → 4 cell Prostrate Thallus → 4 ♂ 10-11μ long 7-8.5μ long → Thallus A
XI (Thallus B)	**Asexual biflagellate (Microzoospores)**				
Juller (1937) *S. subspinosum*	8-9μ 7-8μ	4			Resting Cell → Prostrate Growth → Thallus A
XII (Thallus B)	**Asexual biflagellate (Microzoospores)**				
Cienkowski (1876) *S. tenue*		4-8			Resting Cell → 4 cell Prostrate "sohle" → Thallus A

[3] Rarely ♂ formed from prostrate "sohle" and these germinate directly into Thallus A.



TEXT-FIGURE 3.—Schematic diagram of sexual and asexual cycles and reproduction in *Stigeoclonium* as reported in the literature.

haploid and diploid generations?; or (3) release four quadriflagellate zoospores upon germination (V)—an indication of zygotic meiosis?

Various "cysts" or "resting cells" (II, VI, VIII, XI, XII) have been described as intermediate steps in asexual reproduction. Whether these "resting cells" develop from exclusively asexual zoospores or from gametes which develop parthenogenetically is not completely understood.

"Dwarf-germlings" or "three- to four-celled prostrate thalli" (VI, X)—which could be the same structure, or could be different—have been reported as intermediates in asexual or sexual reproduction.

Pascher (1906c) described aplanospores (called hypnospores by other writers) as "modified microzoospores" (III, IV) which germinated *in situ* to produce very irregular branching of the erect filaments.

Text-fig. 3, p. 27, also shows that two types of *sexual cells* have been reported, namely; *quadriflagellate* (V, from THALLUS A) and *biflagellate* (VII, from THALLUS A; IX, X from THALLUS B). In addition, Pascher (1918) reported amoeboid gametes in *Stigeoclonium*. In spite of repeated attempts, the writers were never able to induce formation of either biflagellate or quadriflagellate *gametes* from actively growing cells or from "*Palmella*-like" (really more like akinetes) cells.

Two types of *asexual* spores have been reported, namely, *quadriflagellate* (I, II, VI from THALLUS A)[29] and *biflagellate* (VIII, XI, XII from THALLUS B). Both Pascher (1906b,c) and Islam (1963) reported that first macrozoospores, and 2–5 days later microzoospores were produced from the same thallus. Klebs (1896) stated that microzoospores were produced in May and June, and that 2% sucrose added to the culture medium increased the number of microzoospores. Several investigators have mentioned that environmental changes apparently induce *Stigeoclonium* plants to form *either* macrozoospores or microzoospores (Godward, 1942; Chang, 1952). The differences in these two types of quadriflagellate zoospores according to Klebs (1896), Godward (1942), and others are summarized in Table 4. More work must be done to confirm the existence of these two types of asexual quadriflagellate zoospores, and the distinctiveness of their behavior. The first six criteria listed in Table 4 are not, in our opinion, consistent or reliable. With regard to criterion 7, the length of time that the "resting cell"[30] persisted before

[29] Really three types if a distinction is made between quadriflagellate macrozoospores and quadriflagellate microzoospores, as most workers have done. The writers are not completely convinced that such a distinction exists. Godward (1942) reported in *S. amoenum* that "microzoospores" were always sexual cells, and died unless fusion occurred. Klebs (1896) never saw fusion of "microzoospores"—after a "resting stage" these cells developed into new plants. Both Godward and Klebs reported direct germination of the "macrozoospores." Fritsch (1935) said that "microzoospores" may be gametes which form a thick-walled resting stage without fusion, and that Klebs' "microzoospores" may indicate this parthenogenetic tendency, or they may be a special kind of asexual spore which is indistinguishable from the others.

[30] The exact interpretation of this term must be clarified. Is the "resting cell" produced by truly asexual cells in the normal process of germination of such cells, or as a result of unfavor-

TABLE 4. *Differences between quadriflagellate macrozoospores and quadriflagellate microzoospores, according to the literature*

	Macrozoospore	*Microzoospore*
1. Difference in the position of the stigma	median, non-protuberant	posterior, more protuberant
2. Size	larger	smaller
3. Number produced in each cell	1	2–4
4. Type of movement	move forward, not "jerky"	don't move forward, rotate in one place, "jerky"
5. Duration of movement	shorter	longer
6. Attraction to light	less attraction	more attraction
7. Type of germination[a]	direct; often, but not always, erect filaments develop before prostrate filaments. May become "resting cell" or "cyst" with thick wall in which further divisions occur (Islam, 1963).	When behaving as a gamete, forms "cyst" or "resting cell"; prostrate filaments develop first when "resting cell" germinates.

[a] cf. Juller (1937) and Chang (1952).

germination was usually not mentioned. Klebs (1896) said that the period varied from a few days to several weeks, and he pointed out the survival value of such spores to the plant. He called this "resting cell" the "transformation product" of the microzoospore. *If* the "resting cell" or "cyst" *always* precedes the germination of asexual microzoospores, and *If* the "resting cell" does persist for a relatively long time, it could hardly have completely escaped our notice. On the basis of this fact we must assume that: (1) all of the quadriflagellate zoospores produced during this investigation were, in fact, macrozoospores; or (2) the microzoospores (or macrozoospores) form "resting cells" only under certain conditions, perhaps to insure survival,[31] and that in the conditions provided by laboratory culture, direct germination always occurred; or (3) there is only one type of asexual zoospore.

In this investigation, zoospores were usually formed in large numbers in the erect filaments of *Stigeoclonium* when the filaments were transferred to fresh medium.[32] Other means of inducing zoospores have been mentioned by Pascher (1906a), Reich (1926), Reynolds (1951), and Hustede (1957). A quadriflagellate zoospore

able conditions? Is it a gamete that develops without fusion perhaps because only one of the two mating types is present? Is it actually a zygote and the investigators failed to observe copulation?

[31] Could this not be equated with the so-called *Palmella* stage?

[32] In preliminary work the writers found several isolates which never would produce zoospores when transferred to fresh medium. The difficulty with *S. helveticum* (isolate S–4) is discussed later, p. 51. Madge (1940) mentioned difficulty in inducing zoospores in some cultures even after transfer to new medium.

(macrozoospore?) was the only type of motile cell ever observed by the writers in this investigation. As a rule, one to two zoospores were released from each cell through a lateral pore or break in the cell wall (Fig. 95, 254). The zoospores were often motile inside the mother cell before release. The release of the zoospores was extremely rapid and after a few minutes entire erect filaments were emptied, only the very thin cell walls remaining (Fig. 99, 243). Longitudinal partition of the cells described by Madge (1940), Godward (1942) or Islam (1963) was not seen; however, diagonal division of the cell contents sometimes occurred as the zoospores were formed (Fig. 98). Very often thin transverse walls were formed so that the cells appeared to be in pairs (Fig. 19, 97). Occasionally the thin transverse walls between the cells broke down before the zoospores were released giving the appearance of four to eight zoospores in a single cell. These zoospores always germinated directly to form new plants.

In addition to the investigators mentioned in the chart accompanying Text-fig. 3, p. 26, accounts of zoospore production have been given by Nägeli (1855), Ström (1921), and Madge (1940).

Berthold (1878) and Gay (1891) mentioned two types of germination (i.e., direct germination without intervening resting stage) of zoospores; Fritsch (1903) added a third type.[33] These three types, as listed by Islam (1963), are:

TYPE I. The zoospore germinates to form an upright filament. From the lowermost cell of the upright filament an irregular branching filament develops which grows over the substratum. The other erect filaments develop from this prostrate branching filament.

TYPE II. The germinating zoospore grows out bilaterally to form a creeping filament which branches several times on the substratum. All erect filaments develop from this prostrate, basal system.

TYPE III. The germinating zoospore forms an upright filament with a basal cell modified for attachment.

The writers noted that the zoospores produced under the controlled conditions of this investigation tended to conform, with some exceptions, to one of the three categories. *In general,* the isolates with well-developed erect systems and small basal systems conformed to type I (Fig. 38), and those with extensive basal systems to type II (Fig. 213). Of the 20 organisms studied, only one, *S. helveticum* (isolate S–4), germinated as type III. The extent to which type of germination of the zoospore may be correlated with definite species, as Islam (1963) suggested,[34] can be definitely established only when the life cycle is clearly known, and when the ques-

[33] Fritsch (1935) said that macrozoospores normally formed basal system first and upright filaments later. However, in some *Stigeoclonium* species erect filaments formed directly from the zoospore.

[34] The first division of the asexual zoospore may be influenced by environmental factors such as light (Peirce and Randolph, 1905).

tions brought out by the work of Juller (1937)[35] and Chang (1952)[36] have been resolved.

The reports of Juller (1937), Godward (1942), and Singh (1954) of the life cycles of *S. subspinosum, S. amoenum,* and *S. farctum* need confirmation. With regard to the life cycle of *S. amoenum,* the account of Singh (1954) directly contradicts that of Godward (1942).

THE PALMELLA STAGE—The so-called *Palmella* stage of *Stigeoclonium,* first described by Cienkowski (1876), is one of the least understood aspects of the life cycle. Whether a *Palmella* stage is an integral part of the life cycle of *Stigeoclonium* (Juller, 1937); merely an incidental phase caused by external environmental factors (Gay, 1891; Klebs, 1896; Tilden, 1896); or, indeed, is misleading terminology and should be abandoned entirely is subject to debate.

Much of the confusion, no doubt, results from the lack of a precise definition of *Palmella* stage as the term is usually applied to *Stigeoclonium.*[37] The term *Palmella* was derived, of course, from the genus *Palmella* of the order Tetrasporales. This genus, however, is subject to much variation of interpretation by phycologists. Very generally, the genus *Palmella* encompasses those algae of the division Chlorophyta which exist as solitary cells or colonies with each cell surrounded by a thick gelatinous sheath which may be either distinct or confluent to form a gelatinous matrix of indefinite size. According to Smith (1950), reproduction occurs by the production of biflagellate zoospores or gametes by the vegetative cells.

The observations of several investigators are reviewed below to illustrate some of the difficulties encountered in the literature regarding a precise understanding of the *Palmella* stage in *Stigeoclonium.*

According to Klebs (1896), the essential characteristics of the *Palmella* stage as described by Cienkowski (1876) are: (a) the disintegration of the membrane connecting the cells of the filament; and (b) the separation of the cells into single cells surrounded by a gelatinous sheath. Although he varied the environment in many ways, Klebs (1896) was unable to induce the formation of a gelatinous matrix in any of his cultures of *S. tenue.* The result, according to Klebs, was always the same. That is, after the cells stopped growing actively, they became spherical

[35] Juller (1937) proposed an alternation of generations for *S. subspinosum* in which a haploid plant alternated with a small (three to four celled) diploid prostrate filament. Quadriflagellate macrozoospores (Text-fig. 3–I) produced by the *n* plant germinated according to type I. Quadriflagellate microzoospores (Text-fig. 3-II) and biflagellate zoospores (Text-fig. 3-XI) germinated according to type II.

[36] Change (1952) proposed a life cycle of *S. subsecundum* involving three types of filaments: one unbranched filament which was the result of germination type III; two types of branched filaments which were identical at maturity—one branched filament was the result of germination type I, and the other type II.

[37] Fritsch (1935), Smith (1950), and Chapman (1962) mentioned the *Palmella* stage without clearly defining the exact morphological attributes of such a stage.

or barrel-shaped and filled with starch and oil. Some dissociation of the spherical cells of the filaments occurred in stronger culture solutions (cf. Livingston, 1900, 1901) and lengthwise division occurred in old cells.

Gay (1891) concluded that several species of *Stigeoclonium* could undergo essentially temporary modifications of the thallus[38] when certain environmental conditions existed.[39] It was suggested that such adaptations made asexual reproduction possible by means other than zoospores.

Tilden (1896) reported that both *Pilinia*[40] and *S. flagelliferum* could form a *Palmella* stage in extremely unfavorable environmental conditions. She described the *Palmella* stage as the condition in which the cells became spherical, thick-walled, divided into two–four parts, and became either solitary cells or small groups of cells surrounded by a single membrane. Such cells, according to Tilden, always formed microzoospores which functioned as gametes.

Livingston (1900, 1901, 1905a,b,c)[41] characterized the *Palmella* stage in *S. tenue* as composed of clumps of spherical cells with a disorientation of the plane of cell division. He said that the cell walls were "somewhat gelatinous," but did not mention any matrix surrounding the cells.

Yatsu (1905)[42] described the cytological differences between the *Palmella* and the filamentous forms of *Stigeoclonium* as follows: *Palmella*—cells spherical, thick-walled, without vacuoles, with larger "chlorophyll granules" and pyrenoid, formed in dry environment; *Filamentous*—cells cylindrical, very thin-walled, with large vacuoles, small "chlorophyll granules" and pyrenoid, formed in wet environment.

Uspenskaia (1936a) mentioned, but did not specifically describe, a *Palmella* condition in *S. tenue* brought about by highly alkaline solutions which were low in nitrogen.

Juller (1937) noted a *Palmella* stage in *S. subspinosum* composed of spherical, thick-walled cells.[43] He said such a stage was an essential part of the life cycle inasmuch as biflagellate gametes were produced from such cells.

[38] Gay listed three such modifications of the thallus: (a) Unmodified vegetative cells of the filament became spherical, formed thick walls, and dissociated. These cells could divide longitudinally or horizontally and sometimes joined to form a "palmelloid mass." Gay did not define "palmelloid mass." (b) Modified vegetative cells (hypnocysts) characterized by thick walls, sometimes formed masses of single cells. These developed, Gay said, under extremely unfavorable conditions. (c) Modified zoospores (hypnospores) enclosed in separate membranes and formed one–several per cell. The hypnospores or hypnocysts mentioned by Gay may be related to the "resting cells" or "cysts" so often mentioned in the preceding discussion of the life cycle of *Stigeoclonium*. Refer to p. 28 of this paper.

[39] Neither Gay nor Tilden (next paragraph) specifically enumerated the conditions which constitute an "unfavorable environment."

[40] According to Tilden, *Pilinia* is a growth form of *S. flagelliferum*.

[41] Livingston's work discussed morphological changes in *Stigeoclonium* induced by increasing the osmotic pressure of the medium.

[42] It should be emphasized that Yatsu used Livingston's cultures for his observations.

[43] Juller's drawings do not clearly show a gelatinous matrix.

Godward (1942) found that old cells of *S. amoenum* often divided longitudinally and, eventually, broke up into single, rounded cells.[44] She said that the whole group resembled "*Palmella* without any formation of mucilage." On drying agar, during the summer, Godward noted that all of the cells of the filaments were occasionally transformed without change in shape into orange akinetes.

Smith (1950, p. 153) said that "*Stigeoclonium* has pseudoparenchymatous *Palmella* stages which can be induced in various ways."[45] He evidently recognized the disorientation of the plane of cell division but not the complete disintegration of the filament into masses of single cells.

Chang (1952, p. 17) used the term "fragmentation"[46] to mean a "type of palmelloid condition which is derived from the filament by the process of fragmentation and which results in cells capable of germination directly into a new filament." This condition, she said, differed from the *Palmella* stage of Cienkowski (1976) or Juller (1937) inasmuch as the cells germinated directly instead of producing microzoospores or gametes. She reserved the terms "resting cell" "cyst" for a type of "palmelloid cell" derived from either macrozoospores or microzoospores which upon germination produced *only* microzoospores.[47] Macrozoospores or microzoospores, according to Chang, encyst under unfavorable conditions to form "resting cells."[48]

Islam (1963, p. 27), referring to the *Palmella* stage, said:

> At this stage the filaments of both erect and prostrate portions may break down and the cells round off, develop a thick wall and may remain singly or in groups very much like protococcoid plants.

From the preceding summary regarding the *Palmella* stage of *Stigeoclonium,* it is interesting to note that: (1) the absence of a gelatinous sheath[49] was specifically mentioned by Klebs (1896) and by Godward (1942); (2) gelatinous sheath was either not mentioned, or not emphasized, by Gay (1891), Tilden (1896), Livingston (1900, 1901), Yatsu (1905), Uspenskaia (1936a), Juller (1937), Smith (1950), Chang (1952), or Islam (1963); (3) all of the illustrations of Cienkowski (1876) do not unquestionably show such a sheath; and (4) too little is known about the life cycle of *Stigeoclonium*[50] to accept such observations as those of Tilden (1896), Juller (1937), or Chang (1952) which relate the *Palmella* stage to the type of reproductive cell which it produces.

[44] This occurred on agar, she said, but not in liquid culture.

[45] A reference to Livingston's work.

[46] According to Chang, "fragmentation" occurred only in the cells of the basal system.

[47] Quite erroneously, in our opinion, she said that such "resting cells" corresponded to the *Palmella* stage described by Livingston (1900).

[48] "Unfavorable conditions" were not specifically enumerated. The writers note the contradiction in these last two sentences.

[49] An attribute, in our opinion, inherent in the use of the term *Palmella.*

[50] Refer to p. 24.

In liquid cultures (age 1–2 months) and, even more often, on agar cultures (age 1–2 months) the writers commonly found cells with the following morphological attributes: spherical or barrel-shaped cells with thick walls sometimes dissociated into short chains, or often even single cells (Fig. 256). A disorientation of the plane of cell division was a common phenomenon (Fig. 167, 237). However, no gelatinous matrix was ever observed surrounding these cells, even the single cells. These attributes do not seem to us to be unusual, or even unexpected, in filamentous algae. Many algae are known to form thick-walled resistant cells, and cell walls are known to thicken as the nitrogen supply is depleted. The accumulation of starch or oil and the rounding up of cylindrical cells is common to many algae upon aging. Division in irregular planes was seen to precede zoospore formation in some actively growing filaments,[51] and it may be that the irregular planes of cell division, so commonly found in old filaments, represent arrested zoospore formation.

Islam (1963, p. 28) said:

> It seems that *Palmella*-cells, hypnospores, akinetes etc. are more or less similar, the only difference being cell-wall thickening and they either produce new plants directly or through the formation of zoospores.

Oltmanns (1922, p. 312) stated that *Palmella* cells of *Stigeoclonium* may also be called akinetes!

In view of the existing confusion regarding an interpretation of such terms as hypnospores, hypnocysts, resting cells, cysts, akinetes, aplanospores, etc., the writers suggest that the terms *Palmella* or *Palmella* stage be supplanted by the term akinete where indicated in discussions of *Stigeoclonium*.

MORPHOLOGY OF THE ERECT SYSTEM—For the purpose of delimiting species in the genus *Stigeoclonium*, previous investigators have always emphasized primarily the characteristics of the erect system. This is, perhaps, understandable because the erect filaments comprise the most conspicuous part of the plant as it is seen and collected in nature. The basal system usually adheres closely to the substratum and is often detached when the plant is collected. Special methods involving living cultures must be employed to study the basal system. The most important keys for determining species (Hazen, 1902; Heering, 1914; Collins, 1928; Islam, 1963; Printz, 1964) are all based on attributes of the erect system. Unfortunately, for the purpose of clearly and precisely delimiting species, the attributes of the erect system used for species characterization are the most variable of the plant.

In the following sections various attributes of the erect system will be discussed in order to measure the value of each as a reliable taxonomic criterion.

Differentiation of the cells composing the main axis of the filament—Various

[51] According to Islam (1963, p. 29), "Vegetative cells may divide in different planes at the time of zoospore-production."

investigators have mentioned that in most *Stigeoclonium* species all of the cells of the main axis are similar in size and shape. Lateral branches, produced from these undifferentiated cells, are usually composed of cells which are about the same size as those of the main axis. Some workers call this the primitive type.

In other *Stigeoclonium* species, the cells of the main axis are differentiated into groups of four to eight, short narrow (or short globose) cells irregularly placed among the long, wide cells which form most of the filament. These long, wide cells usually have thicker walls, and a small parietal, band-like chloroplast which fills only the center of the cell. Only the short, narrow (or short, globose) cells produce lateral branches. The cells of the branches are usually much narrower than those of the main axis. The presence of modified branch-producing cells is restricted to the cells of the main axis. There is usually no differentiation in the size of the cells of the primary branches from which the secondary branches originate. Some workers call this the advanced type.

If the condition is, in fact, as stable and as clearly evident as outlined above, the genus *Stigeoclonium* contains entities which in differentiation and specialization of the cells of the main axis parallel the transition described in the genera *Draparnaldia* and *Draparnaldiopsis*.[52]

Islam (1963, p. 50), attempting to "classify the species into several groups based upon a particular character," was the first investigator to stress the differentiation of the branch-producing cells of the main axis. Accordingly, in his key, all *Stigeoclonium* species were separated into three main groups, as follows:

Group I. Species with main axis and primary branches similar; no specialized or modified cells present on main axis producing branches.

Group II. Transition between Group I and Group III. Species which show more differentiation between the cells of the main axis—some cells being long and some short; branches commonly produced by the shorter cells.

Group III. Species in which the main axis usually includes two types of cells—long, usually not producing branches; small and short, usually producing lateral primary branches.

Although the length of the cells of the main axis varied considerably in most of the cultures studied in this investigation,[53] the writers do not consider that any of the cells were specialized or modified for branch production. Branches were often produced from small cells, but these cells were not smaller than, and often not as small as, other cells of the filament which did not produce branches (Fig.

[52] In *Draparnaldiopsis,* tuft-like lateral branches are produced from groups of small cells irregularly arranged among the longer cells in the main axis. No specialized branch-producing cells are found in *Draparnaldia*; the main axis consists of long cells some of which produce the tuft-like lateral branches.

[53] Refer to the next section, "Size of the cells of . . .," p. 37, for a complete discussion of this question.

149). Long and small cells in the same culture often produced branches (Fig. 150). An extreme condition such as that in which smaller, narrower cells arranged in groups of four–eight among longer ones, as illustrated in *S. amoenum* (Hazen, 1902; Godward, 1942; Islam, 1963), was never encountered in this study.[54]

On the basis of a careful examination of the descriptions and illustrations of various workers (particularly those of Kützing, 1853; Berthold, 1878; Wolle, 1887; Hazen, 1902; Islam, 1963), as well as observations of the isolates used in this study, the writers are convinced that the presence of specialized, branch-producing cells in the main axis is *clearly* evident in only a few *Stigeoclonium* species, and, for this reason, would hesitate to place quite as much emphasis on the trait as has Islam (1963).

Only three of the eight species listed by Islam (1963) in Group III,[55] in our opinion, *definitely* have specialized branch-producing cells; namely, *S. amoenum*,[56] *S. flagelliferum*,[57] and *S. nudiusculum*. Islam's description (1963, p. 129) of *S. nudiusculum* was based on Kützing's herbarium specimen, No. 7, *Draparnaldia nudiuscula*, collected September, 1834. Islam (1963, p. 131) said that this specimen resembled *S. flagelliferum* except in type of branching and in the presence of multicellular colorless hairs. Hazen (1902) stated that *S. flagelliferum* could be a form of *S. amoenum*, differing only in the larger diameter of cells (14–18μ for *S. flagelliferum* and 11.5–16μ for *S. amoenum*) and in the possession of "attenuated, setiferous terminal branches."[58] Apropos to a discussion of difference in branch tips of these three species, it is interesting to note that Godward (1942, p. 293) reported that in *S. amoenum* "hairs were not a feature of the plant when in a young and vigorous condition, but generally appear when vegetative growth is at an end." Based on these statements, and subject to confirmation in cultural studies, it is our opinion that this group may contain only one, and certainly no more than two, distinct entities.[59]

[54] Godward (1942) mentioned that differentiation of cells of main axis and branching habit were obscured to some extent in plants brought from nature to laboratory culture. However, plants arising from zygotes produced in culture resembled very closely plants growing in nature.

[55] Islam listed eight species in Group III: *S. amoenum, S. flagelliferum, S. nudiusculum, S. lubricum, S. elongatum, S. tenue, S. pachydermum,* and *S. paihiae*. The writers choose to defer judgment on *S. pachydermum* and *S. paihiae* which have been described from recently isolated collections.

[56] Islam (1963) considered *Myxonema ventricosum* Hazen and *S. insigne* Nägeli to be identical and placed them as a variety of *S. amoenum*.

[57] Tilden (1896) mentioned that in *S. flagelliferum* Kütz. branches were often, but not always, produced from groups of small cells. Tilden's identification was questioned by Hazen (1902) and Pascher (1906c). Hazen said that Tilden should have erected a new species rather than "stretch" the description of *S. flagelliferum* to fit her isolate.

[58] Hazen's and Islam's use of the term "setiferous," not altogether clear to the writers, is interpreted to mean an outgrowth of the cell wall which does not enclose a nucleus.

[59] While the writers may accept *S. amoenum* as distinct, *S. flagelliferum* and *S. nudiusculum* are considered to be the same.

The differentiation of the branch-producing cells of the main axis is less distinct in *S. lubricum*, and even less so in *S. tenue* and *S. elongatum*. Hazen (1902, p. 197) says that *S. lubricum* may be considered as a "standard point of departure" for comparison of the other forms of the group with "small branch-bearing cells," and that *S. tenue* is a much finer and somewhat simpler form, and might well be placed in the ancestral line of *Myxonema lubricum*."[60] Kützing (1853) did not illustrate specialized branch-bearing cells in either *S. lubricum* or *S. tenue*,[61] and only hinted at their presence in *S. elongatum*. Berthold (1878) was the first to illustrate clearly the differentiated branch-producing cells in *S. lubricum*, but both Hazen (1902) and Islam (1963) emphasized their presence in this species which Collins (1928) reported as the most common *Stigeoclonium* on the eastern coast of the United States. Islam (1963) says that in *S. tenue* branches usually develop from "angular cells" which are smaller than the others. Hazen (1902) does not stress this trait in his description of *S. tenue*. The writers do not fully understand Islam's placement of *S. elongatum* in Group III. Kützing's illustration (1853) showed some branches originating from single small cells but some from long cells also. Neither Hazen (1902) nor Collins (1928) mentions any differentiation of the branch-bearing cells of *S. attenuatum*, yet Islam (1963, p. 105) says:

> Hazen's descriptions for his new species *S. attenuatum* (Hazen) Collins are more or less the same as what [sic] Kuetzing gave for *S. elongatum* (1849) and there is little doubt that these two species are the same.

Islam (1963) reports that *S. elongatum* looks like *S. tenue* var. *tenue* except for differences in width of filaments and sharp-pointed branch tips.[62] ". . . As a matter of fact, it can be said that just as *S. tenue* is a miniature *S. amoenum*, so *S. elongatum* is a miniature *S. flagelliferum*."

In summary of the preceding discussion, the writers suggest that comparative cultural studies would reveal a few (possibly only three–four) distinct entities in the six species listed above. The real challenge, however, would be to locate living plants for such studies which could be *definitely* assigned to one, and only one, of the above-mentioned species.

Size (length and width) of the cells of the main axis—Cell division in the erect filaments of *Stigeoclonium* is intercalary or diffuse, rather than localized (Fig. 14, 61, 141).[63] Therefore, the length of the cells of the main axis, as well as that of

[60] Refer to p. 17 for a discussion of Hazen's use of *Myxonema* for *Stigeoclonium*.

[61] According to Islam (1963), Kützing's illustration of *S. lubricum* does not represent the species as it is understood today. Hazen (1902) cites Kützing's illustration of *S. tenue* as the source of much of the misinterpretation of the species.

[62] The writers do not consider these differences to be sufficient to distinguish *S. elongatum* as another species.

[63] Vischer (1933) described the growth of *Caespitella* as entirely apical. The writers found apical growth on agar, but not always in liquid, cultures of the *Caespitella*-like isolates (Ca 421, 18–3, 10–2) studied in this investigation. Apical growth was also found in the cells comprising

the cells of branches, in any actively growing plant, will vary between rather widely separated extremes (Fig. 14–16, 139). In addition, the length of the cells is also influenced by the rate of growth (i.e., the rapidity of cell division) which is largely dependent on external factors, such as temperature, light, and nutrients. Uspenskaia (1936b) found that the concentration of nitrate in the medium and light intensity greatly influenced the average length, but not necessarily the width, of the cells of *S. tenue*. Evidently, intensity of light, rather than the amount of nitrate, was more limiting; increasing the nitrate at low light intensity resulted in smaller cells than when the concentration of nitrate was increased at high light intensity. Vischer (1933) found length of cells in *S. tenue* somewhat dependent on pH.

The width of the cells of the main axis is, perhaps, a more constant character than the length. However, older cells are wider than younger ones, and the ratio of length and width of the cells is a factor of the age of the culture (Fig. 59, 69).

By measuring a large number of cells, an investigator can, at best, ascertain only maximum and minimum width, or an average based upon these two extremes. Cell length is usually expressed as a function of width; for example, cells two–eight times as long as wide. Even under ideal conditions, it is difficult to distinguish between the main axis, the primary and the secondary branches, and impossible to be sure that all extremes of width and length have been included.

The difficulties encountered when an investigator attempts to compare the cell size of specimens which can only be assumed to be related to other similar specimens, or in which the age or rate of growth can not be determined, or in which only a small portion of the thallus can be seen, are too obvious for further comment. Islam (1963) stated that on numerous occasions he found the length and width of cells to be greater than, or less than, the measurements previously reported for the same specimens.

It appears to us that the separation of one species from another based on differences of a few microns in either the length or width of the cells is clearly suspect, if not completely to be disregarded.

Shape of cells of the main axis—Most of the cells of the isolates observed in this study were cylindrical, and little constricted at the partition wall in the young, actively growing stage (Fig. 59). With aging (or during zoosporogenesis), the cells became more barrel-shaped and constricted at the partition wall (Fig. 69, 97). The cells of the branches were always more cylindrical than those of the *older* portions of the thallus. Sometimes within a single culture, the shape of the cells of the filaments varied greatly, i.e., some filaments had very pronounced constrictions at the partition wall, and others had slight or no constrictions (Fig. 137–140). It would have been easy to suppose the filaments belonged to two different organ-

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the basal systems of all of the *Stigeoclonium* cultures; i.e., the cells of the basal systems did not exhibit the extreme size variation found in the erect filaments.

isms had not each culture been derived from the isolation of a single filament. Descriptions of the shape of cells of the main axis given by various investigators often make distinctions as to shape of cells "below" compared to those "above." In view of the variation in a single culture, the writers have concluded that such detailed descriptions are misleading and not representative of all members of the species.

Formation of a mucilage layer around the cells of the erect filaments or basal cells—Although the literature states that the erect filaments of *Stigeoclonium* are usually surrounded by a thin layer of mucilage (thicker in some species),[64] no such mucilage formation could be detected with India ink or methylene blue in any actively growing culture under the conditions of this investigation. However, the tips of the rhizoids often were surrounded by a "sticky substance" (Fig. 85). After periods of 2–4 months in poor light, a few cultures (notably *S. tenue,* Var I) were surrounded with mucilage, although never to the extent found in *Chaetophora.* Fritsch (1903) mentioned mucilage formation in cultures of *S. variabile* left in poor light.

Several writers (Wolle, 1887; Tilden, 1896; Madge, 1940) have stated that early growth stages of some species of *Stigeoclonium* appear to be much like *Chaetophora.* Islam (1963, p. 40) described this similarity: ". . . being surrounded by heavy gelatinous sheath, but later as the plant grows, the filaments break through the mucilage matrix." Islam (1963) implied that low temperature may be responsible for the production of mucilage, since he observed a thin layer of mucilage surrounding *S. subsecundum* growing under ice. The writers have had several isolates of *Chaetophora* in culture for some time, and have observed that the erect filaments often resemble those of *Stigeoclonium.* However, *Chaetophora* cultures always produce a thick mucilaginous sheath, clearly detectable in India-ink preparations, and *Stigeoclonium* does not.

A "brownish-red" substance surrounding the cells of the basal system, similar to that reported by Fritsch (1903), Chang (1952), and Juller (1937), was seen by us in cultures in BBMP enriched with soil supernatant (3:1) before isolations were made, but never in unialgal, bacteria-free cultures in either BBMPTB$_{12}$, or BBMP plus soil supernatant (3:1). The writers believe that the "brownish-red" substance —whatever it is—is not a secretion of the plant, but probably a deposit by some other organism at the base of the *Stigeoclonium* plant.

Thickness of the cell walls of the main axis—Although Islam (1963) stated that the cell walls of several species of *Stigeoclonium* were noticeably thickened,[65] and

[64] Islam (1963, p. 39–40) states that in some species (*S. lubricum, S. longipilum, S. nudiusculum, S. pachydermum*) the mucilage layer is comparatively thicker than in other species. This needs to be confirmed.

[65] Islam was unable to determine the age or state of growth of most of the organisms he observed, since for the most part he based his study on the comparison of herbarium specimens.

that this attribute is of some importance for species identification, the writers observed no unusual amount of thickening of the cell walls of actively growing filaments under the conditions of this investigation. However, as the plants ended the period of active growth, the thickening of the cell walls increased (Fig. 136). Often the thickness of the cell wall varied considerably in a single culture. The tendency of the cell walls to become more thickened in old cultures was observed both in liquid and on agar.

Internal structure of the cells of the erect thallus—The internal structure of the cells of all the isolates used in this investigation was similar, and typically ulotrichaceous. Each cell, except those of the hairs or the rhizoids,[66] had a single, parietal, girdle- or band-shaped chloroplast which contained from one to several pyrenoids (Fig. 59, 141). Within the same culture, the chloroplast of actively growing cells often occupied the whole cell, or a portion of the length (usually the middle) of the cell (Fig. 14–16).[67] No significant difference in chloroplast structure was evident. As the cells aged and accumulated starch and oil, the outline of the chloroplast could no longer be accurately distinguished (Fig. 207). Uspenskaia (1936a) showed that the amount of starch in the cells was a factor of the brightness of the light. In bright light, as the concentration of nitrate in the thallus of *S. tenue* decreased, the amount of starch increased, and the appearance of the chloroplast changed.

Although preliminary efforts have been made (Islam, 1963) to distinguish between species of *Stigeoclonium* according to chloroplast type,[68] the results of our investigation show that, at least as far as the isolates considered here are concerned, no separable entities exist.

Formation of branches and branching habit of the erect thallus—The branching habit has always been considered to be one of the most important attributes for the identification of species in the genus *Stigeoclonium*. Through the years, however, many investigators (Berthold, 1878; Gay, 1891; Klebs, 1896; Fritsch, 1903; Vischer, 1933; Uspenskaia, 1936a,b; Butcher, 1950; Reynolds, 1951; Islam, 1963; Abbas and Godward, 1963) have pointed out that the orientation of

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The species that Islam mentioned as having thick walls were the following: *S. pachydermum* (4.5–7 μ), *S. nudiusculum* (7 μ), *S. flagelliferum* (1.5–2.5 μ), *S. lubricum, S. paihiae, S. variabile* (under unfavorable conditions) and one variety of both *S. amoenum* and *S. tenue*.

[66] A nucleus and remnants of chloroplasts could be seen in cells of hairs and in rhizoids. This fact lends evidence to the association of these structures with branches (refer to p. 24, 48).

[67] A chloroplast occupying only the middle of the cell was more evident in older cells, although this was not an absolute rule.

[68] Islam (1963) emphasized that in all *Stigeoclonium* species the younger cells of the main axis and cells of the smaller branches usually have a ulotrichoid chloroplast. However, with reference to the older cells of the main axis and the larger branches, he noted several types of chloroplast differentiation, and tentatively related them to certain species. Until such time as cultural studies reveal that these distinctions exist, the writers feel that they must be discounted.

branches is extremely variable. The observations of several of these investigators, which include some conflicting results, are summarized in Table 5.

Based on the results of our own studies, which included observations of *Stigeoclonium* in both nature and in culture, it seems clear that the pattern of branch ing can be described only in broad and general terms. To do otherwise would be, to say the least, misleading. The practice of past investigators, i.e., of precisely delimiting the branching habit, all too often from a single collection, or even on the basis of a single thallus, and expecting every member of the species to conform very closely to this type, has resulted in an unrealistic number of species, and to a need, often expressed in a recent work (Islam, 1963), to determine the exact range of variation within the species.

The following terms are those used most extensively in the literature to describe the type of branching in a species: alternate, pseudodichotomous, opposite, dichotomous, whorled, unilateral (secund), irregular, approximate (i.e., branches arising from each of several adjacent cells), and unbranched.

Although Islam (1963) considered pattern of branching to be an important specific attribute and differentiated between several closely related species on this basis alone, his descriptions of various *Stigeoclonium* species illustrate that he recognized the diversity of branching types in a single species. The following description of the branching habit of *S. longipilum* (Islam, 1963, p. 62) is representative of those given for other species and will illustrate the point:

> . . . [B]ranching more or less dichotomous, or alternate, rarely opposite above; less branching below; more and repeated branching above giving a bushy or tufted appearance (i.e., each main filament remains unbranched up to some distance from the base and then branches repeatedly either dichotomously or alternately to give a radiating or spreading appearance). . . .

The writers take exception, not with Islam's description of alternate, dichotomous, or even opposite branching in the same organism, but rather with his precise placement of the branches, ". . . less branching below; more and repeated branching above, . . ." etc.

The exact placement of either primary or secondary branches is, in our opinion, so much a factor of environment and age that one can define them only in terms of these conditions, and even then some variation exists in a single culture.[69]

[69] The writers call attention to the following two quotations from Islam (1963, p. 30–31): ". . . Thus it seems that branching habit or growth is significantly dominated by chemical nutrients in the environment. Therefore, for a taxonomic evaluation of a species, in culture at least, one has to determine the proper concentration of the medium in which the plant may fully express itself. In nature also it seems that these plants express themselves fully only when a 'proper' concentration of chemical nutrient is available."

. . . . . .

". . . Thus it seems that it is equally risky to identify each and every collection from nature and

TABLE 5. *Partial summary of the effect of various environmental factors on the branching pattern of* Stigeoclonium

Klebs (1896) (*S. tenue?*)	Found that the degree of branching depended on the concentration of nutrients in the culture solution. Klebs obtained long, sparsely branched, narrow filaments in a 0.2% nutrient solution; however, each cell of the filament grew into a branch in a 0.5–1.0% nutrient solution.
	Found, both in nature and in the laboratory, that light determined the orientation of the branches. Unilateral light induced branch formation on the more strongly lighted side and caused branches that had already been formed to show strong positive phototropism. Light from all sides induced branches in several directions.
Butcher (1950) (*S. nanum*)	Found long, unbranched filaments in excess PO_4, abundantly branched filaments in excess NO_3, and short, branched filaments in excess Mg.
Uspenskaia (1936b) (*S. tenue*)	Found that increasing the amount of NO_3 in the culture medium in constant light intensity (i.e., "natural light") caused the branching pattern to change from "normal" (described as "well-branched") to an excessively branched, "dwarfish" plant.
Vischer (1933) (*S. helveticum*)	Found that light determined the orientation of the branches—branches formed on side toward unilateral light source. More branching in older filaments, especially near edge and surface of the medium.
Reynolds (1951) (*S. farctum*)	Found that different branching patterns result from different culture media. Found *S. farctum* to have an erect system of colorless hairs in "old neglected biphase cultures," but in Godward's solution the same plant produced abundant erect filaments.
Islam (1963) (in general)	Found that the direction of the light strongly influenced the orientation of branches both in nature and in culture; specifically, unilateral light resulted in secund branching.
Abbas and Godward (1963) (*S. amoenum* principally)	Found that branching increased with lengthening the period of illumination (optimum period 18 hr, longer periods harmful). Orientation of branches depended upon the direction of light. Increase in number of branches as temperature increased up to 20°C. Branching promoted by decrease in nitrate and suppressed by increase. Branching suppressed by blue light.

Under the conditions of this investigation, it may be stated that the type of branching most often present in all of the isolates was *alternate* (Fig. 198); however, in each species, configurations which could be interpreted as pseudodichotomous, dichotomous, and opposite occurred. Fritsch (1935, p. 251) says of the erect system of *Stigeoclonium*: "The branches arise from the top of the parent cell and evection is frequent." Through the process of evection, as described by Fritsch, alternate and dichotomous branching may sometimes be confused.[70] Unbranched filaments, secund branching, irregular branching, and approximate branching occurred regularly in the same culture (Fig. 194–200). Although the branching pattern in a particular species *may be* predominantly *alternate, opposite,* or, to a lesser degree *whorled*,[71] the degree of branching, the placement of the branches, and the orientation of the branches is so variable due to conditions that a precise description is not possible. One will search in vain, either in nature or in culture, for organisms which will *exactly* fit the descriptions of each species as given by Islam (1963) or other investigators.

The method of formation of the primary and secondary branches followed a similar pattern in all of the isolates studied by the writers. In general, the branches arose from cells of the main axis or primary branches through an enation of the cell contents, followed by the formation of a transverse wall which separated the branch from the cell which produced it (Fig. 22–24). Branches often originated from the upper end of the parent cell just beneath the septum (Fig. 87) but sometimes also from the middle of the cell (Fig. 91). The lateral branches in the prostrate filaments of the basal systems were formed in a similar manner.

Occasionally in all of the isolates used in this study, branches were formed from a small cell in the filament (Fig. 150).[72] The cell contents more rarely divided diagonally prior to branch formation (Fig. 62–64).[73] In older cultures, particularly

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also to rely on any culture where concentration of chemical nutrients, light, temperature, etc. could be the regulating factors on the growth of the plants. *Therefore one must use his personal judgment to decide which plant-collection should be taken as reliable for species identification.*" [Emphasis added.]

[70] According to Fritsch (1935) evection is the process whereby a branch, first formed at a wide angle from the main axis (alternate type), becomes upwardly displaced through localized growth of the membrane of the parent cell beneath the branch. Subsequently, the branch lies on the same level as the main axis and results in the appearance of a dichotomy. Although dichotomous branching was often seen in these cultures, it was difficult in many cases to determine whether the branching was truly dichotomous (i.e., the result of the division of an apical cell) or pseudodichotomous (i.e., the result of evection).

[71] Whorled branching was not observed in any of the cultures in this investigation. Islam (1963) did not mention whorled branching as the primary type in any species. The extent of whorled branching in *Stigeoclonium* is yet to be determined.

[72] Discussed on p. 34.

[73] This phenomenon was also observed subsequent to zoospore formation (p. 30). The writers do not think diagonal partitioning normally precedes branch formation.

2-month-old agar cultures,[74] cells of the filaments divided to produce four cells (Fig. 167). If the filaments of such cultures were moistened with nutrient solution, branches formed from each cell, including those that had divided. This resulted in an irregular mass of branches.

Nature of the tip of the erect filaments—The confusion in terminology with regard to "hairs" in various algal genera was discussed by Möbius (1892) and Huber (1892). Huber pointed out that in the Chaetophoraceae the "hairs" are elongated vegetative cells and include a nucleus. He suggested that the term ("hair"[75] (= *"poil"* or *"pilum"*) be applied to this condition. He further suggested that the term "bristle" (= *"soies"* or *"seta"*) be applied to hyaline appendages which are simple outgrowths of the cell wall and which do not include a nucleus. Fritsch (1935), who agreed with the distinction of terminology proposed by Huber, said the term "hair" should be applied to the Chaetophoraceae, and "bristle" or "seta" to the members of the Coleochaetaceae or Chaetosphaeridaceae. Throughout this paper, the writers have used the term "hair" in the sense of Huber and Fritsch.

Such words as the following are used in species descriptions by Islam (1963) and others to describe the nature of the tip of the erect filaments: pointed, attenuate, setiferous,[76] flagelliform, blunt, acute, long unicellular colorless hair, and long multicellular colorless hair.

Islam (1963) considered the nature of the tip of the erect filaments of "fully-developed" *Stigeoclonium* plants to be "fairly uniform," "not the same in all species," and useful "to a limited extent" in separating species. In fact, he did distinguish between several species on the basis of the nature of the branch tip alone. However, many investigators (Klebs, 1896; Fritsch, 1903; Pascher, 1906b; Cholnoky, 1929; Vischer, 1933; Uspenskaia, 1936b; Godward, 1942; Reynolds, 1951; Hustede, 1957; Abbas and Godward, 1963) have presented evidence (which Islam recognized) as to the variability of the tips of the erect filaments. A partial summary of the work of some of these investigators is presented in Table 6. Based on the results of our own study, and supported by the evidence of previous workers, the writers question the validity of the nature of the tip of the erect filaments as a taxonomic criterion. Long multicellular colorless hairs (Fig. 135) were found in all of the isolates studied by the writers except four, namely: S–4, Ca 421, 10–2, and 18–3.[77] Often, in the same culture, where differences in environment could

[74] Refer to p. 34.

[75] Preserving the distinction between "unicellular hair" and "multicellular hair."

[76] Both Hazen (1902) and Islam (1963) often used the term "setiferous" in such expressions as "setiferous without a hair." Since neither worker explained whether he meant that the branch tip was "bristle-like" or had a "unicellular hair," the meaning is rather obscure. During this study, no condition was ever seen in any isolate which could be equated with "setiferous" in the sense implied by Huber or Fritsch.

[77] S–4 is Vischer's isolate of *S. helveticum*. Ca 421, 10–2, and 18–3 correspond very closely to his descriptions of *Caespitella pascheri* and the writers consider that they are identical.

not be considered a factor, hairs were noted at the tips of the erect filaments of some plants, whereas the tips of others might be described as "blunt," "acute," "pointed," or "attenuate" (Fig. 193). It is our opinion that these conditions represent intermediate stages which may ultimately result in the formation of a multicellular hair.

Islam (1963, p. 39), in attempting to group several species of *Stigeoclonium* according to the nature of the branch tip, stated: "A well-developed plant always tends to produce tips characteristic of that species-group. . . ." The writers wonder how much he used ". . . his personal judgment to decide which plant-collection should be taken as reliable for species identification."[78]

MORPHOLOGY OF THE BASAL SYSTEM OF THE THALLUS (INCLUDING A DISCUSSION OF RHIZOIDS)—Fritsch (1935, p. 251) stated:

> Since the prostrate system of *Stigeoclonium* accommodates itself to all irregularities of the substratum, it is not easily detached or studied, and is therefore in the majority of cases very incompletely known.

According to Islam (1963, p. 38):

> . . . because the prostrate system often is not so conspicuous and because the cells are more variable[79] this portion of the thallus is not considered much for taxonomic differentiation.

The manner in which *Stigeoclonium* has been studied in the past has made the accumulation of reliable information about the morphology of the basal system difficult, if not impossible. Previous investigators have, at best, given it only cursory attention.

Descriptions of the basal systems of 28 species of *Stigeoclonium* recognized by Islam (1963) are presented in Table 7. Islam's illustrations of the different types of prostrate systems (Pl. 2, Fig. 2–3; Pl. 3, Fig. 4–6; and Pl. 40, Fig. 4–8) do not, in our opinion, accurately convey the diversity which exists in the genus.

After a careful comparative study, both in nature and in culture, of 17 isolates of *Stigeoclonium* and of three *Caespitella*-like organisms, the writers have concluded that the morphological attributes of the prostrate system are considerably more stable than those of the erect system and that they provide a firmer basis for species separation than do the attributes of the erect system.[80] This position contrasts sharply with that of Islam (1963) who reported that the same species of

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Vischer (1933) noted that multicellular hairs were not formed in *S. helveticum* or *C. pascheri* in Knop's medium or in sterilized pond water. However, such hairs were formed in "frisch Sumpfwasser." See Table 6, p. 46.

[78] See footnote 69, p. 41.

[79] No valid evidence is offered to support this statement.

[80] The writers do not, of course, state that conditions *could not be devised* to change the morphology of the basal system. Nevertheless, in the controlled conditions of culture, the variability of the basal system never approached that of the erect system.

TABLE 6. *Partial summary of the effect of various environmental factors on the nature of the branch tip in* Stigeoclonium

Abbas and Godward (1963) (*S. amoenum* principally)	Found hair formation promoted by decrease in nitrate and suppressed by increase in nitrate.
Pascher (1906b) (*S. nudiusculum*)	Found no hairs in running water, but hairs formed in still water.
Klebs (1896) (*S. tenue*?)	Rarely found hairs in the spring, under "jet of water" where the alga was collected. However, very long, multicellular hairs formed in laboratory in still water.[a]
Reynolds (1951) (*S. farctum*)	Found low NO_3 in the culture medium favored the reduction of the erect system to colorless hairs. Attributed change from green erect filaments to colorless hairs to adaptation of the plant to poorer nutrient conditions.
Uspenskaia (1936b) (*S. tenue*)	Found that increasing the intensity of the light and the concentration of nitrate in the medium suppressed the development of hairs.
Cholnoky (1929) (*S. tenue*)	Found fewer hairs on plants in running water than in standing water.
Vischer (1933) (*S. helveticum* and *C. pascheri*)	Found more hair formation in standing pond water than in flowing water. Varying the amount of phosphate in unsterilized pond water to obtain different pH caused differences in hair production in young plants. Hairs produced most often in young germlings; seldom found in older plants. Sterilization of pond water, tap water, or 1/3 Knop's solution almost completely inhibited hair formation.
Hustede (1957) (*S. falklandicum*)	Hairs often produced when 1% glucose added to the medium, seldom produced in inorganic medium under controlled conditions.
Godward (1942) (*S. amoenum*)	Exhaustion of content of medium and cessation of growth caused terminal cell to produce hair.

[a] Klebs asks which element of "running water" prevented hair formation. He thought the most likely explanation was that friction caused the hairs to break off. He considered the increase in nutrient salts in culture medium as a factor; however, in a 0.5–1.0% concentration of nutrient salts, most of the branch tips were sharp-pointed without hairs like the alga in the stream.

TABLE 7. *Descriptions of the basal systems of 28* Stigeoclonium *species as summarized by Islam (1963)*

S. farctum	Cushion-like prostrate part, cells more or less angular, compact, nearly isodiametric, forming pseudoparenchymatous or monostromatic base. In culture LB 439 from Indiana University Culture Collection of Algae, Islam saw only the monostromatic prostrate thallus, and no disc.
S. variabile	Pseudoparenchymatous or monostromatic prostrate part of round or angular cells; cells 15–20μ in diameter.
S. nanum	Loose, parenchymatous disc composed of angular or isodiametric cells, or of monostromatic creeping filaments, every cell producing an erect filament. Cells of prostrate filaments longer than erect filaments.
S. aestivale	Erect filaments *radiating* from a palmelloid base or creeping filaments composed of isodiametric cells or a mass of narrow, downward-growing filaments and rhizoids.
S. thermale	Profuse erect filaments from creeping prostrate base with rhizoids.
S. subsecundum	Prostrate part of thallus lacking or may consist of short, inflated, more or less isodiametric cells. Rather long rhizoids from the cells above the base.
S. amoenum	Prostrate part absent, attached to substrate by profuse rhizoids.
S. longipilum	Not well developed, but consisting of profuse, dense rhizoidal filaments. Rhizoids produced from basal cells of erect filaments, or from base of cells at place of branching.
S. fasciculare	Erect filaments radiating from palmelloid or rhizoidal base.
S. tenue	Prostrate part may be palmelloid or with profuse rhizoids.
S. lubricum	Prostrate thallus creeping, often reduced and attached by rhizoids.
S. nudiusculum	Rhizoids from basal cells.
S. Nelsonii	Prostrate part lacking.
S. carolinianum	Prostrate part apparently lacking, attached to substratum by rhizoids.
S. curvirostrum	Well developed, attached to substratum by rhizoids, cells of prostrate part round or globular, 8–15μ wide, 11–17μ long.
S. pachydermum	Basal part lacking, attached to substrate by numerous basal, downward-projecting rhizoids.
S. elongatum, S. pusillum, S. setigerum, S. paihiae, S. lebelii, S. biasolettianum, S. segarae, S. flagelliferum, S. subuligerum, S. stagnatile, S. protensum and *S. helveticum*	Basal systems not mentioned.

Stigeoclonium could produce "different types of prostrate systems depending upon the various environmental factors in addition probably to the nature of the substratum."[81]

Emphasis on the prostrate system as a taxonomic criterion will not facilitate identification of natural collections of *Stigeoclonium,* for the basal system is not easily observed unless the alga is cultured; however, knowledge of the basal system is nonetheless indispensable.

It is often stated that in some *Stigeoclonium* species the basal system is lacking and the plant is attached to the substratum by profuse rhizoids originating from the lower cells of the erect thallus. Gay (1891) described such rhizoids as branches "adapted to a new function." Islam (1963) stated that although any species may, under certain conditions, form rhizoids, some species invariably do so, and others do so only occasionally. In only one of the *Stigeoclonium* isolates studied in this investigation were rhizoids the sole means of attachment (Fig. 26).[82] However, rhizoids were abundantly produced from the cells of the basal prostrate filaments in many of the cultures (Fig. 78, 121).[83] Although the writers noticed no change in the gross morphology of the different basal system types in either the control medium (BBMPTB$_{12}$), organic medium (3 parts BBMP: 1 part soil supernatant), or in nature, the extent to which rhizoids were formed from the cells of the prostrate filaments was somewhat variable.

Berthold (1878) and Fritsch (1903) reported that rhizoids were not found as often in nature as "in culture." The writers also found fewer rhizoids on plants grown on glass slides in nature.[84] However, in laboratory culture, at least, it was clear that rough substrates repress rhizoid formation whereas smoother ones enhance it. The contribution of Peirce and Randolph (1905, p. 329) concerning the effect of the substrate on the development of the holdfast of freshwater algae[85] is relevant here:

A uniform though slightly rough surface, like that of polished glass, will induce the formation of very symmetrical holdfasts. Coarsely or irregularly roughened surfaces

[81] Islam's only evidence for this statement seems to be gained from his study of culture LB 439, from the Culture Collection of Algae, Indiana University, Bloomington, Indiana, which is labeled *S. farctum* Berthold. Refer to page 63 for a complete discussion of this organism.

[82] Isolate S–4 (#441, Indiana University Culture Collection of Algae), Vischer's *S. helveticum,* a very "*Ulothrix*-like" *Stigeoclonium,* produced a few rhizoid-like growths from the cells of the erect filaments. These rhizoids were never very profuse, as had been described for *S. amoenum,* etc.

[83] Islam (1963) said rhizoids grow from the lower sides of the prostrate thallus. In this investigation they were clearly extensions of the terminal cells of the prostrate thallus.

[84] The writers were less successful in transplanting organisms with small prostrate systems to the natural environment than organisms which produced larger prostrate systems.

[85] The work of Peirce and Randolph concerned *Oedogonium* especially.

induce the formation of irregular holdfasts. Extremely smooth surfaces fail to induce the formation of holdfasts, unless perhaps of the most rudimentary sort.[86]

Although the smooth surface of the glass slide, no doubt, induced the formation of "very symmetrical" prostrate systems in the *Stigeoclonium* and *Caespitella*-like organisms studied in this investigation, the extent to which the smooth substrate accounted for the increase in rhizoid production is yet to be determined.

Rhizoids produced from the cells of the basal filaments appeared morphologically identical to the rhizoids which were occasionally produced from the cells of the erect filaments. In general, both were multicellular filaments composed of slender, long cells. The proximal cells of the rhizoid often contained a small chloroplast; the distal ones, however, were often colorless, and often bent, crooked, curved in different directions, i.e., somewhat corkscrew in shape (Fig. 129).

Cienkowski (1876) and Gay (1891) described a condition in which the prostrate filaments of several plants actually grew together or coalesced to form an "aggregate" basal system. The writers observed that zoospores often came to rest near one another, sometimes even tending to clump, and the prostrate filaments produced from such germinating zoospores grew adjacent to one another (Fig. 191). However, it was always possible to trace the individual growth at some place on the slide and no evidence was observed that any physical joining ever occurred between different plants. Tilden (1896) said that it was not uncommon for filaments developed from spores germinating near one another to become entwined and eventually to form portions of the same thallus, but that the thallus could also originate from a single spore. This confirmed the writers' observations.

Observations of the Erect and Prostrate Thallus in Nature and in Culture

In the morphological discussion which follows, the 17 isolates of *Stigeoclonium* and the three *Caespitella*-like isolates have been grouped according to the nature of their basal systems in view of the reliability of this attribute as emphasized above.

The seven basal system types (comprising three major divisions) represent the mature morphological expressions of the organisms concerned. Young plants are less readily distinguishable unless the growth of the basal system is followed to maturity.

Grouping the isolates according to the nature of their basal systems may be summarized as follows:

GROUP I. *Basal system consisting of short, sparsely produced rhizoids*
Scattered rhizoid-like filaments developing from the base of the erect filament or

[86] All of the organisms studied in this investigation produced only rudimentary basal systems on the surface film of liquid medium or on agar. No rhizoids were produced in either case.

laterally from other cells of the erect filament..... *S. helveticum* (S–4) (Fig. 26)

GROUP II. *Basal system consisting of a branching filament*

Here four subcategories may be distinguished:

A. A small filament from which prostrate lateral branches of restricted growth develop. These may rebranch. The ends of prostrate filaments are sometimes, but not always, rhizoidal..... *S. aestivale* (HP 4, Var 5, 8–3) (Fig. 44, 48, 50)

B. A small filament from which prostrate lateral branches of restricted growth develop. Numerous rhizoids develop from the cells of the basal system, so that the latter appears to be a mass of rhizoids..... *S. subsecundum* (19–11–V) (Fig. 78, 79)

C. An extensive prostrate filament of indeterminate (unlimited) growth from which lateral branches arise in an irregular manner. These lateral branches, which rebranch, often become as extensive as the main filament. The cells of the basal system are globular or barrel-shaped (i.e., cylindrical and constricted at the partition walls)..... *S. tenue* and *S. pascheri*

Here two subcategories may be distinguished:

1. The ends of the lateral prostrate filaments are often very slender and exhibit a "corkscrew"-like appearance as they extend into colorless rhizoids. The cells of the erect system are clearly different from those of the basal system and the erect filaments can easily be distinguished from the basal system..... *S. tenue* (6–1D, Var 1, Gold, 19–1–E) (Fig. 108, 113, 121, 122)

2. The ends of the lateral prostrate filaments seldom exhibit a "corkscrew"-like appearance. The cells of the basal system are spherical or subspherical. The erect system is usually, but not always, of limited growth (i.e., not well developed) and often is inconspicuous, but may be very long. In aerated cultures these plants form discrete colonies on the slide. The ends of the basal filaments always tend to become detached from the substratum, and proliferate upward. Without close examination, these extensions of the basal cells can be confused with the erect system so that one might interpret them as two very different types of erect filaments..... *S. pascheri* (18–3, Ca 421, 10–2) (Fig. 152, 155, 156)

D. A branching filament with a predominant main filament from which lateral prostrate filaments of limited growth arise. These lateral branches rebranch, sometimes, but not always, forming a very compact filament, almost a disc, with globular or round cells..... *S. variabile* (Jo, 6–15, 6–23) (Fig. 184, 190)

GROUP III. *Basal system consisting of a pseudoparenchymatous disc*

A short, prostrate filament of restricted growth from which prostrate lateral filaments develop in an irregular manner, the laterals rebranching and becoming as long as the main filaments. These lateral filaments develop adjacent to each other and form a

pseudoparenchymatous *Coleochaete*-like disc....*S. farctum* (5–3C, 5–3F, 19–5–V, 7–17) (Fig. 227)

Stigeoclonium helveticum Vischer

(Isolate S–4)

This organism, designated by the code S–4, is Vischer's *S. helveticum* var. *maius*.[87] Vischer (1933) described the species *S. helveticum* and, on the basis of size alone, established two varieties, namely, *S. helveticum* var. *maius* and *S. helveticum* var. *minus*. The writers, who have had both of these isolates in culture throughout this investigation, agree with Islam (1963) that the difference in size does not warrant retention of the two varieties.

MORPHOLOGICAL OBSERVATIONS[88]—*Zoospores and germling stage*—Under standard conditions, this organism rarely produced zoospores. In fact, motile cells were never observed[89] and germinating zoospores only twice. The germination of the zoospores corresponded to type III.[90] Following a transverse division, the upper cell of the germinating zoospore formed an erect filament; the lower cell formed a *Ulothrix*-like holdfast, which subsequently developed into a small rhizoid for attachment.

Mature basal system—The long, erect filaments were loosely[91] attached to the glass slide by short rhizoids from the lower or middle cells (Fig. 26). These rhizoids were never very profuse. Vischer (1933) demonstrated the presence of a small amount of mucilage around the rhizoid tips.

Erect system—Actively growing cells (2–3 weeks or younger)[92] were cylindrical with no constrictions at the partition wall (Fig. 14–17, 20, 21, 26, 28, 29). Older cells (1 month) were more rounded and constricted (Fig. 18, 19). Cells of 2-month-old $BBMPTB_{12}$ agar cultures had very thick walls (Fig. 27), and might properly be called akinetes. When fragments of older filaments were transferred from agar to a liquid medium, the cells usually produced new filaments directly. Often each cell produced a new branch (Fig. 25, 28).

The width of the cells of actively growing, erect filaments was from 8 to 12μ (average 10μ) (Fig. 14–16, 20). Cell length was extremely variable, from 15 to 83μ (Fig. 14, 16), in this culture.[93]

[87] S–4 is isolate No. 441 from the Culture Collection of Algae, Indiana University, Bloomington, Indiana (Starr, 1964). Vischer originally deposited this organism in the Culture Collection of Algae and Protozoa, The Botany School, Cambridge, England (No. 477/1).

[88] Based on culture work only.

[89] Vischer (1933) was also unable to determine the number of flagella present in the zoospore.

[90] Refer to page 30.

[91] The plants were usually detached as the slide was removed from the Erlenmeyer culture flask.

[92] Throughout the following discussion, "actively growing" refers to cultures 2–3 weeks old or younger; "older" refers to cultures more than 1 month old.

[93] Vischer (1933) reported length up to 10× the diameter.

Actively growing filaments were usually sparsely branched (Fig. 28, 29). However, older cultures in liquid medium or on agar were more profusely branched[94] (Fig. 25, 27).

Branching was mostly alternate (Fig. 24), rarely approximate (Fig. 30), or opposite from swollen, probably old cells. The branches developed from an enation of the cell contents, followed by the formation of a cell wall[95] (Fig. 22–24, 29).

The branch tips of the actively growing filaments were always blunt (Fig. 21), without a hair. The terminal cell of the erect filament of a germinating zoospore often extended into a rather hyaline, pointed tip. However, even in the germlings, fully developed multicellular hairs were never seen.[96]

Colony characteristics—A flat, vermiform or wavy colony was characteristic of a one-month-old culture grown on 1.5% $BBMPTB_{12}$ agar[97] (Fig. 31). As a rule, the filaments grew close to the surface of the agar; sometimes a *very few, short,* erect filaments occurred in the center of the colony.

Color upon aging—On 1.5% $BBMPTB_{12}$ agar in 100- × 13-mm culture tubes, the color of the colony was as follows:[98] 1 month, grass-green; 2 months, grass-to-light green; 4 months, light-green.

Stigeoclonium aestivale (Hazen) Collins (Emend.)
(Isolates 8–3, Var 5, HP 4)

MORPHOLOGICAL OBSERVATIONS—*Zoospores and germling stage*—One to two quadriflagellate zoospores were produced in each cell of the erect filaments and released through a pore in the wall. Zoospores were produced in great numbers 1–2 days after the filaments had been transferred to fresh media (Fig. 54). The zoospores, immediately after coming to rest, measured from 10 to 12μ in length and from 4 to 5μ in width (in isolate 8–3 from 8 to 10μ in width).

Germination began as soon as the zoospores had come to rest and conformed to type I.[99] The zoospore first formed an upright filament (Fig. 32, 35, 38). An irregularly branching prostrate filament formed through unilateral (Fig. 32, 33, 35, 36, 38, 41), and usually subsequent bilateral (Fig. 33, 34, 36, 37, 39, 40, 42),

[94] Vischer (1933) reported that under favorable conditions, in flowing culture medium and on agar, the erect filaments were sparsely branched and resembled *Ulothrix*. He found more branching in old filaments when the culture flask was full, especially near the edges and on the surface of the medium.

[95] Islam (1963, p. 76) stated that the branches form "by evection from any place of the cell" and "the branching habit is the only characteristic feature." The writers found the formation of branches in *S. helveticum* not unlike that in several other species of *Stigeoclonium*. The "*Scytonema*-like" branching described by Islam was not seen.

[96] In 1/3-strength Knop's medium Vischer (1933) found no hairs in old filaments (endings were blunt), and hairs soon disappeared completely from the germlings.

[97] Vischer (1933) obtained similar results on 1/3-strength Knop's agar.

[98] Vischer (1933) also noted the absence of carotin formation in old cells.

[99] Refer to page 30.

germination from the lowermost cell of the erect filament, i.e., the original zoospore. Other erect filaments developed from the prostrate filaments (Fig. 38–40).

Mature basal system—The mature basal system was a small filament from which prostrate lateral branches of restricted growth developed (Fig. 44, 50, 51, 53, 55, 56, 58). These prostrate lateral branches often rebranched (Fig. 50, 55). The ends of the prostrate filaments were sometimes, but not always, rhizoidal in culture (Fig. 45–49). Rhizoids developed from the ends of fragments of the erect system (Fig. 52). Cells of the erect system[100] also formed rhizoids which anchored the tips of the erect filaments to the glass substrate.

Erect system—Young, actively growing cells were cylindrical with little constriction at the partition walls (Fig. 48, 59, 60). However, with age the cells became more rounded. Thick-walled, spherical cells were produced after 2 months on $BBMPTB_{12}$ agar (Fig. 69–73). These cells (akinetes) were filled with starch and oil. The terminal cells of the filament sometimes formed "knobs" (Fig. 73, 74). Although all of the cells in isolate Var 5 did not form akinetes, some invariably did so (Fig. 72). The cells of the filaments often tended to dissociate, especially in cultures 8–3 and HP 4 (Fig. 69, 75). These dissociated cells were not surrounded by any sort of mucilaginous matrix and, therefore, no "*Palmella* stage"[101] was ever observed in any of these cultures.

The cell width and length of actively growing erect filaments was as follows:

	Width	Length
Isolate 8–3	5–10μ	12–25μ
Isolate Var 5	5μ	5–25μ
Isolate HP 4	4–6μ	8–25μ

The erect system was extensively developed (Fig. 48, 58). Young cultures contained long, unbranched (Fig. 65), or sparsely branched filaments (Fig. 43). At 3–4 weeks, the filaments were often very branched, both in liquid cultures (Fig. 51, 66, 67) and on agar surfaces (Fig. 70).

The type of branching was as follows: unbranched (Fig. 47, 48, 65), alternate (Fig. 43, 51, 57), unilateral, and irregular (Fig. 66, 67). Branch formation often followed a diagonal division of cell contents (Fig. 62–64).

The tips of the branches were blunt (Fig. 68); sharp-pointed, almost dagger-like (Fig. 68); or with multicellular colorless hairs (Fig. 43, 57, 74). The latter were common in young cultures of isolate 8–3 (Fig. 43) and in 3-week- to 1-month-old cultures of Var 5 (Fig. 57). Hairs were rarely produced in isolate HP 4—usually only in very old agar cultures (Fig. 74).

Colony characteristics—A 1-month-old culture on 1.5% $BBMPTB_{12}$ agar was flat and vermiform or wavy (Fig. 77). *Schizothrix*-like tufts or matted (caespitose) filaments (Fig. 76) often occurred near the center of the colony.

[100] Especially near the surface of the culture medium.

[101] Throughout, references to the *Palmella* stage are *sensu* Cienkowski (1876).

Color upon aging—On 1.5% BBMPTB$_{12}$ agar in 100- × 13-mm culture tubes, the color of the colony was as follows:

	1 month	2 months	4 months
Isolate 8–3	grass-green	light-green	yellow-green
Isolate Var 5	grass-green	light-green	yellow-orange-green
Isolate HP 4	grass-green	light-green	light-green

Stigeoclonium subsecundum (Kütz.) Kützing
(Isolate 19–11–V)

MORPHOLOGICAL OBSERVATIONS—*Zoospores and germling stage*—Usually one to two quadriflagellate zoospores were produced in each cell of the erect filaments and released through a lateral pore. However, on one occasion, when this alga was brought into the laboratory after 2 weeks in the Blanco River, San Marcos, Texas, two quadriflagellate zoospores were produced in each cell and released in a vesicle (Fig. 95).[102]

As in *S. aestivale* (isolates 8–3, Var 5, HP 4), the zoospores germinated as soon as they stopped moving and produced an erect filament from the basal cell of which lateral prostrate filaments subsequently developed also. Other erect filaments were produced from the prostrate growth (type I).[103]

Mature basal system—The mature basal system consisted of a small filament from which prostrate lateral branches of restricted growth developed (Fig. 81, 82). Numerous slender, spreading rhizoids developed from the cells of the basal system, so that the latter sometimes appeared as a mass of rhizoids (Fig. 78–80).[104] Rhizoids also developed from cells of the erect filaments—often from small, barrel-shaped cells (Fig. 84, 85), but also from cylindrical cells (Fig. 86). Rhizoids formed from the ends of fragments of erect filaments (Fig. 94).

Erect system—Cells of the erect filaments of actively growing cultures were cylindrical with little constriction at the partition wall (Fig. 78, 84–91). A few of the cells at the lower portion of the erect filaments were shorter and barrel-shaped (Fig. 85). Cells of 2-month-old BBMPTB$_{12}$ agar cultures were slightly more restricted but did not form thick-walled, globose, akinete-like cells (Fig. 92, 93). No "*Palmella* stage" was seen.

Actively growing cells of the erect filaments varied from 4 to 10μ in width and from 10 to 35μ (sometimes 50μ) in length.

The erect system was very extensive (Fig. 79, 81, 83). Both branched (Fig. 78, 87) and unbranched (Fig. 88) filaments occurred in young cultures.

The type of branching was variable. Alternate branches were often found (Fig. 78, 84–86, 89, 90). Well-formed branches appeared to be dichotomous. Secund

[102] Microzoospores or macrozoospores? They did not form a resting stage.

[103] Refer to page 30.

[104] Rhizoids were less frequently observed in nature than in culture.

branching also occurred (Fig. 87) and unbranched filaments were present (Fig. 88).

The branches were usually formed at the top of the cells of the erect filaments, near the septa, by enation of the cell contents, followed by the formation of a transverse wall (Fig. 87, 90). Branches were also formed from the middle of the cell (Fig. 91).

In culture, the tips of the filaments were blunt (Fig. 91) or sharp-pointed (Fig. 86–88); multicellular hairs were rarely found. In nature the tips of the erect filaments were blunt (Fig. 85), sharp-pointed (Fig. 90), or with multicellular colorless hairs (Fig. 84, 85, 89).

Colony characteristics—A 1-month-old culture on 1.5% BBMPTB$_{12}$ agar was vermiform at the extreme edges with horn-like, or *Schizothrix*-like tufts, or occasionally matting (caespitose) in the center of the colony (Fig. 96).

Color upon aging—On 1.5% BBMPTB$_{12}$ agar in 100- × 13-mm culture tubes, the color of the colony was as follows: 1 month, dark-green; 2 months, grass-to-light-green; 4 months, light-green.

Stigeoclonium tenue (Ag.) Kützing (Emend.) (Isolates 6–1D, Gold, 19–1–E and Var I)

MORPHOLOGICAL OBSERVATIONS—*Zoospores and germling stage*—The zoospores were quadriflagellate, produced one to two (Fig. 97, 98) in each cell and released through a pore in the cell wall (Fig. 99). The zoospores usually germinated according to type I (Var I, 19–1–E, Gold—Fig. 104); occasionally, type II (6–1D—Fig. 100–103).[105] The first erect filament developed from the zoospore (Fig. 103, 104). A prostrate filament developed first from one side of the zoospore (Fig. 100, 101, 104) and, subsequently, equal bipolar prostrate development occurred (Fig. 100, 102, 103).

Mature basal system—The mature basal system was an extensive, prostrate filament of indeterminate (unlimited) growth from which lateral branches arose in an irregular manner (Fig. 105, 106, 111, 112, 115, 121, 125). These branches, which rebranched, often became as extensive as the first filament (Fig. 107, 108, 110, 121–123). The cells of the basal system, especially those near the center, were globular or barrel-shaped—i.e., cylindrical and constricted at the partition wall (Fig. 107, 109, 114, 115, 121, 122). The ends of the lateral prostrate filaments were often very slender and exhibited a "corkscrew"-like or wavy appearance as they extended into colorless rhizoids (Fig. 108, 113–115, 121–124, 129). These rhizoids usually developed for some distance from the main filaments of the basal system (especially in isolates 6–1D and Var I). In addition to the rhizoids formed at the ends of the prostrate filaments, rhizoids often developed from the cells of the erect filaments, although never very profusely. Rhizoids also developed from the

[105] Refer to page 30.

ends of fragments of erect filaments (Fig. 116). Sometimes the rhizoidal ends became detached from the glass substrate and grew upward. At the surface of the culture medium, the basal system was smaller and more compact (Fig. 119, 120). The cells of the basal system, both in nature and in culture,[106] formed a compact filament of akinete-like cells (Fig. 117, 118, 126, 127).[107] This was the form seen in the Blanco River at San Marcos, Texas (Fig. 128). It is probable that this compact filament resulted from the dissociation and subsequent loss of some of the cells of the longer lateral branches, and particularly the rhizoids.

Erect system—There was *always* a distinct erect system developing from the cells of the basal system and, in contrast to the situation usually found in *Stigeoclonium pascheri*,[108] these erect filaments could easily be distinguished from those of the basal system. The erect filaments were extensively developed and often very long, sometimes twisted in rope-like fashion, on the surface of the culture medium. As in the other isolates studied in this investigation, branching was more common in older cultures. Filaments in young, actively growing cultures were usually unbranched.

The cells in such cultures were cylindrical, with little constriction at the partition wall (Fig. 139, 141, 142). The older portions of the thallus were sometimes, but not always, composed of cells which were barrel-shaped, and deeply constricted at the partition wall—especially in isolates Var I and 19–1–E (Fig. 134, 137, 138, 140). At 2 months on $BBMPTB_{12}$ agar, the cells became spherical and developed thick walls (Fig. 136, 143, 144). These cells, akinetes, were filled with starch and oil and often dissociated into single cells, especially in isolate 6–1D. The end cells were often globular (Fig. 144). No *Palmella* stage was ever seen in any of these cultures.

The average length and width of actively growing cells of the erect filaments of each of the isolates in this group was as follows:

	Width	Length
Isolate 6–1D	4–7–(10μ)	(5)–16–20μ
Isolate Var I	4–8–(10μ)	(6)–20–22–(30μ)
Isolate Gold	5μ	(5)–16–22–30μ
Isolate 19–1–E	4–5μ	10–20–(30μ)

The following types of branching were found: unbranched filaments (Fig. 126); alternate (Fig. 130, 133, 137, 138); unilateral (Fig. 130); and dichotomous or pseudodichotomous (Fig. 131).

The tips of the erect filaments were blunt (Fig. 142), sharp-pointed (Fig. 111), and occasionally with multicellular colorless hairs (Fig. 131, 132, 135). Multicellular hairs were very long in isolate Var I (Fig. 135). Although multicellular

[106] However, only in cultures enriched with soil supernatant.

[107] Somewhat like isolate Jo, *S. variabile*. Refer to page 59.

[108] Refer to page 57.

hairs were rarely observed in isolates Gold and 19–1–E under standard conditions, they were often observed in isolate 6–1D. Sometimes in isolates 6–1D and Var I the ends of the erect filaments were crooked or "corkscrew"-shaped and connected the ends of erect filaments to the glass substrate (Fig. 133).

Colony characteristics—One-month-old cultures on 1.5% BBMPTB$_{12}$ agar were matted or caespitose (Fig. 145). Sometimes the colony was vermiform at the *extreme* edge, or with a *few* tufts in the center.

Color upon aging—On 1.5% BBMPTB$_{12}$ agar in 100- × 13-mm culture tubes, the color of the colony at one, two, and four months was as follows:

	1 month	2 months	4 months
Isolate 6–1D	light-green	light-yellow-green	yellow-green
Isolate Var I	light-green	light-yellow-green	yellow-green
Isolate Gold	grass-green	light-yellow-green	orange-green[109]
Isolate 19–1–E	grass-green	light-green	yellow-green

Stigeoclonium pascheri comb. nov.

(Isolates Ca 421, 18–3, and 10–2)

Isolate Ca 421 is Lewin's isolate designated *Caespitella* sp.[110] The writers have had *Caespitella pascheri* Vischer[111] in culture during this investigation, and Lewin's isolate (Ca 421) and both of the writers' isolates (18–3 and 10–2) appear to be identical to it. After careful study, we have concluded that the genus *Caespitella* erected by Vischer (1933) does not differ sufficiently from the genus *Stigeoclonium* Kützing to warrant retention. For reasons that will be discussed later,[112] the writers have abandoned the genus *Caespitella* Vischer and have designated the three organisms so labeled as *Stigeoclonium pascheri.*

MORPHOLOGICAL OBSERVATIONS[113]—*Zoospores and germling stage*—Two quadriflagellate zoospores, similar to those in other species of *Stigeoclonium*, were produced in each cell of the erect filaments or in the cells of the detached basal filaments of the thallus. The zoospores often germinated *in situ* (Fig. 163). Germination of the zoospores corresponded to type I (Fig. 146) or to type II (Fig. 147).[114] In the same organism, either unilateral (Fig. 146) or bilateral (Fig. 147, 148) development of the basal filaments occurred from the zoospore.

[109] Occurred only once. Culture usually dead by this time.

[110] No. 421 from the Culture Collection of Algae, Indiana University, Bloomington, Indiana. Lewin originally deposited this isolate in the Culture Collection of Algae and Protozoa, The Botany School, Cambridge, England (No. 410/2).

[111] No. 320 from the Culture Collection of Algae, Indiana University, Bloomington, Indiana. Vischer originally deposited this isolate in the Culture Collection of Algae and Protozoa, The Botany School, Cambridge, England (No. 410/1).

[112] Refer to page 73.

[113] Morphological observations based on cultural studies only.

[114] Refer to page 30.

Mature basal system—The mature basal system was an extensive prostrate filament of indeterminate (unlimited) growth from which lateral branches developed in an irregular manner (Fig. 148, 151, 152). These lateral prostrate branches, which rebranched, often became as extensive as the first filament (Fig. 153, 154, 159) and the basal system was characterized by its very spreading nature (Fig. 155). The cells of the basal system, especially in older cultures, were spherical or barrel-shaped—i.e., cylindrical and constricted at the partition walls (Fig. 155, 157, 158, 160, 161). Rhizoids were seldom produced[115] from the ends of the lateral prostrate filaments, although the ends of the filaments were sometimes slightly "corkscrew"-shaped (Fig. 151, 158). The ends of the basal filaments became detached from the substratum, and proliferated upward (Fig. 156, 161). Unless the culture was closely examined, these upward extensions of the basal cells were easily confused with the erect filaments.

Erect system—There was much variation in the length of the erect filaments (Fig. 148, 151). Often the erect system was not well developed, and the true erect filaments were inconspicuous (Fig. 156, 162, 163). As mentioned previously, the upward proliferations of the detached basal filaments were easily confused with the true erect filaments. However, sometimes the erect filaments were very long (Fig. 148), even forming a rope-like growth on the surface of the culture medium.[116] Isolates 18–3, 10–2, and Ca 421 formed discrete colonies on the slide in aerated cultures (Fig. 2, 156).[117]

The actively growing cells of the erect system were cylindrical and little constricted at the partition walls (Fig. 149, 150, 162). Older erect filaments were more barrel-shaped. Very round, thick-walled cells—akinetes—formed at 2 months on $BBMPTB_{12}$ agar (Fig. 164–167). The terminal cell of the filament was often bulbous (Fig. 165). The contents of some of the cells had divided into two to four parts (Fig. 166, 167). These thick-walled cells, when transferred to fresh medium, germinated directly into new filaments. No *Palmella* stage was ever found in any of these cultures.

The average width and length of cells in the *erect filaments* of actively growing cultures were as follows:

	Width	Length
Isolate 18–3	4–6μ	12–26–(36μ)
Isolate Ca 421	5–10μ	10–25μ
Isolate 10–2	(3)–5–6μ	12–23–(30μ)

Actively growing erect filaments were unbranched (Fig. 148, 162), or profusely

[115] Never to the extent found in *S. tenue*. Refer to page 55.

[116] Vischer (1933) mentioned that the basal system and the erect system sometimes exhibited little differentiation in his cultures, and sometimes more so.

[117] Even when the same medium was employed, this phenomenon was less evident or absent entirely in unaerated cultures.

branched in an alternate, sometimes almost dichotomous, pattern (Fig. 149, 150). Unilateral branching also occurred.

No multicellular hairs were ever observed in any of these cultures—10–2, 18–3, or Ca 421. The branch tips were blunt (Fig. 150) or slightly pointed (Fig. 149). The writers were unable to induce hair formation in any medium, even in young germlings.[118]

Colony characteristics—A 1-month-old culture on 1.5% $BBMPTB_{12}$ agar was matted or caespitose (Fig. 168). The colony (especially isolate 10–2) was rarely composed of curved bundles of filaments at the *extreme edge*, or a few *Schizothrix*-like tufts occurred at the center.

Color upon aging—On 1.5% $BBMPTB_{12}$ agar in 100- × 13-mm culture tubes the color of the colony was as follows:

	1 month	2 months	4 months
Isolate 18–3	light-green	light-green	yellow-orange-green
Isolate Ca 421	light-green	light-green	light-green
Isolate 10–2	grass-green	light-green	yellow-green

Stigeoclonium variabile (Nägeli) Islam

(Isolates 6–15, 6–23, Jo)

MORPHOLOGICAL OBSERVATIONS—*Zoospores and germling stage*—One to two quadriflagellate zoospores were produced in each cell of the erect filaments and released through a lateral pore. Zoospore germination appeared to conform to either type I or type II.[119] After coming to rest, the zoospores often formed a *short,* branching prostrate filament from which erect filaments subsequently developed (type II—Fig. 169, 171). However, frequently the first erect filament developed from the zoospore prior to the development of the prostrate filament (type I—Fig. 172, 173 at arrow). Equal bilateral prostrate development usually occurred from the zoospore (Fig. 169–172); occasionally, however, the development was primarily unilateral (Fig. 173).

Mature basal system—The basal system was a branching filament with a dominant main filament from which lateral prostrate filaments of limited growth developed (Fig. 169–172, 174–181). These lateral prostrate filaments rebranched (Fig. 171, 172, 180, 183, 184, 186, 189), usually forming a very compact basal system of round, globular, akinete-like cells (Fig. 182, 185, 187, 188, 190, 191).[120] Rhizoids were rarely observed in these cultures (see Fig. 172, however).

[118] Vischer (1933) mentioned that hairs were found more often in young germlings than in older plants, and in unsterilized pond water more often than in sterilized pond water or Knop's medium.

[119] Refer to page 30.

[120] Although this phenomenon appeared in one other culture (isolate 6–1D, p. 55), it was not regularly and consistently found under standard conditions.

Erect system—Young, actively growing cells were cylindrical with little constriction at the partition wall (Fig. 186, 196–198); older cells were more constricted (Fig. 199, 201, 203, 204). After 2 months on $BBMPTB_{12}$ agar the filaments were much-branched with round, thick-walled cells—akinetes (Fig. 207). The terminal cells of the filament were often bulbous (Fig. 207). No *Palmella* stage was ever found in these cultures.

The cell width and length of actively growing erect filaments were as follows:

	Width	Length
Isolate 6–15	4–5μ	6–8–10–12–(20μ)
Isolate 6–23	5–6μ	8–10–12–13–(30μ)
Isolate Jo	(5–6)–12–14μ	12–14–16–(24μ)

Extreme variation was found in both the length and the degree of branching of the erect filaments. Erect filaments were both very long (Fig. 175) or very short (Fig. 188, 205, 206); both unbranched (Fig. 175, 183, 188, 190, 194, 206) or profusely branched (Fig. 195, 199, 201). These erect filaments often (Fig. 187, 188), but not always (Fig. 186), appeared to be radiating from the cells of the basal system.

The following types of branching of the erect filaments were observed: alternate (Fig. 186, 192, 196–198, 200); unilateral or secund (Fig. 192, 193, 195, 199–204); irregular (Fig. 195); approximate (Fig. 192, 196, 200); and rarely, opposite (Fig. 196) or dichotomous (?) (Fig. 192).

The branch tips were either very pointed or acute (Fig. 186, 192, 194–198, 200); blunt (Fig. 193, 199–203); or with long, multicellular colorless hairs (Fig. 177, 183, 188, 193, 194, 205, 206.)

Colony characteristics—One-month-old cultures on 1.5% $BBMPTB_{12}$ agar were characterized by the predominant *Schizothrix*-like tufts[121] which covered the major part of the colony (Fig. 208, 209). The extreme center of the colony was *occasionally* matted or caespitose, and the extreme edge *occasionally* vermiform. These expressions were subordinate to the characteristic tuft-like appearance (Fig. 209).

Color upon aging—On 1.5% $BBMPTB_{12}$ agar in 100- × 13-mm culture tubes, the color of the colony at 1, 2, and 4 months was as follows:

	1 month	2 months	4 months
Isolate 6–23	grass-green	light-green-orange on top	yellow-orange-green
Isolate 6–15	dark-green	grass-light-green	yellow-green
Isolate Jo	dark-green	grass-green	light-green

[121] The tufts sometimes appeared more "horn-like"—a response to condensed moisture on the inner surface of the culture-dish lid (Fig. 210).

Stigeoclonium farctum Berthold
(Isolates 5–3C, 5–3F, 19–5–V, 7–17)[122]

MORPHOLOGICAL OBSERVATIONS—*Zoospores and germling stage*—Usually one, sometimes two, quadriflagellate zoospores were formed in each cell of the erect filaments and released through a lateral pore in the wall (Fig. 211, 243). Two types of zoospore germination were found.[123] *Typically*, the zoospore underwent bipolar germination to form a prostrate filament which branched several times on the substratum (Fig. 212–1, 213, 214, 216, 217). All erect filaments developed from this prostrate, basal portion (type II).[124] *Less typically*, erect filaments developed as soon as the zoospores germinated and the prostrate filaments developed from the lowermost cell of the erect filament, i.e., from the original zoospore (type I[125]—Fig. 212–2, 215).

Mature basal system—The mature basal system was a short, prostrate filament of restricted growth from which prostrate lateral filaments developed in an irregular manner, rebranching themselves, and becoming as long as the main filament (Fig. 214, 217, 218, 219). These lateral filaments developed adjacent to each other and as they grew, formed a pseudoparenchymatous, *Coleochaete*-like disc[126] (Fig. 221–230). Islam (1963, p. 53) questioned whether the "compact *Coleochaete*-like base as shown by Berthold[127] developed from a single zoospore or from many. . . ." From culture work it was clear that the disc-like basal system did, indeed, develop from a single zoospore (Fig. 214, 227). However, it was not uncommon for two discs to grow together (Fig. 226, 228). The pseudoparenchymatous disc was found both in nature and in culture.

Rhizoids usually did not develop from the terminal cells of the basal system. However, downward-growing rhizoidal filaments often developed from the erect filaments in older cultures (Fig. 244, 245). Rhizoids also developed from the ends of fragments of the inoculum which failed to produce zoospores (Fig. 219).

Erect system—Young, actively growing cells were cylindrical (Fig. 233, 234, 236). As the culture aged, the cells became slightly more barrel-shaped (Fig. 231, 232, 239). In 2-month-old BBMPTB$_{12}$ agar cultures, the cells became rounded, increased in width, and their walls thickened to form akinete-like cells which were

[122] Isolate 7–17 showed some morphological and physiological differentiation from the other isolates in this group. These differences are indicated in the descriptions below.

[123] Refer to page 30.

[124] Usual form of zoospore germination in isolates 5–3C, 5–3F, 19–5–V, and 7–17.

[125] Less common type of germination in all isolates. No morphological difference in the zoospores could be detected. Berthold's illustration (Text-fig. 5, p. 76) offers another possible explanation for apparent type-I germination in *S. farctum*.

[126] Isolate 7–17 sometimes (Fig. 220), but not always (Fig. 222), failed to form a complete disc-like basal system—at least within the time interval allowed for examination. There was, however, extensive proliferation of the lateral prostrate branches.

[127] Refer to Text-fig. 5, p. 76.

filled with starch grains (Fig. 237, 238). Enlarged bulbous cells were often located at the ends of such filaments (Fig. 238), and the contents of the cells were divided into two, three or four parts (Fig. 237). No *Palmella* stage was ever observed.

The width and length of actively growing cells of the erect filaments were as follows:

	5–3C	5–3F	19–5–V	7–17[128]
width	5–8μ	5–7μ	5–7μ	3–6μ
length	8–10μ	5–8–(10)μ	8–10–(13)μ	10–14–(30)μ

The erect filaments developed from the basal cells and many erect filaments were often produced (Fig. 227). The erect system was often quite extensive (isolates 7–17 and 19–5–V), or less so (isolates 5–3C and 5–3F).

The following types of branching were observed: unbranched for considerable distance from the base (Fig. 220, 223–226, 240, 241, 243); alternate (Fig. 226, 235); dichotomous[129] (Fig. 234); unilateral or secund (Fig. 231–233, 239, 241); irregular[130] (Fig. 242, 244, 245); and approximate (Fig. 235, 239, 241).

The branch tips were blunt (Fig. 232, 233, 236), pointed (Fig. 226, 239, 240), or with multicellular colorless hairs (Fig. 225, 235, 243).[131] Under standard conditions isolates 5–3C, 5–3F, and 19–5–V usually produced multicellular hairs only on unbranched erect filaments near the surface of the medium. Isolate 7–17 produced multicellular hairs abundantly on both branched and unbranched filaments.

Colony characteristics—One-month-old cultures on 1.5% $BBMPTB_{12}$ agar exhibited the following colony characteristics:

Isolates 5–3C, 5–3F, and 19–5–V: *Schizothrix*-like tufts covering the entire surface of the colony. These algae formed discrete colonies on the agar surface (Fig. 246, 247).

Isolate 7–17: matted, with distinct *Schizothrix*-like tufts. This alga did not form discrete colonies on the agar surface (Fig. 248). The *extreme* edge of the colony was sometimes composed of curved bundles of filaments.

Color upon aging—On 1.5% $BBMPTB_{12}$ agar in 100- × 13-mm culture tubes, the color of the colony at 1, 2, and 4 months was as follows:

	1 month	2 months	4 months
Isolate 5–3C	dark-green	grass-light-green	yellow-green
Isolate 5–3F	dark-green	grass-light-green	yellow-green
Isolate 19–5–V	dark-green	grass-light-green	light-green
Isolate 7–17	dark-grass-green	light-green	yellow-green

[128] Note slightly greater cell length of isolate 7–17.

[129] Especially in isolate 7–17.

[130] Islam (1963) suggested that irregular branching may be the result of *in situ* germination of aplanospores. The writers did not observe a rounded protoplast within the cell to indicate that aplanospores had been formed.

[131] Erect system often reduced to multicellular colorless hairs in organically enriched medium (i.e., 3 BBMP:1 SS—Fig. 224) or in nature, perhaps in response to lower concentration of nitrogen.

Stigeoclonium sp.

(Isolate S–5)

In addition to the four isolates—5–3C, 5–3F, 19–5–V, and 7–17—considered in the preceding section and definitely identified by us as *Stigeoclonium farctum* Berthold, it is necessary to discuss one other isolate in this group, isolate S–5,[132] which had been previously identified by other workers as *Stigeoclonium farctum* Berthold. The responses of this organism, both morphologically and physiologically, are too different from those of other strains of *S. farctum* to be explained as variations. The writers believe that this isolate, S–5, is not *S. farctum,* but some other species. The disposition of this organism must await further study. Unfortunately, the use of this organism and LB 440[133] has led Islam (1963) to an erroneous conclusion concerning the basal system of *S. farctum.* (See his illustration, Pl. 39, Fig. 6, 7—Text-fig. 4.) Islam (1963, p. 38) stated:

The disc-like prostrate part of *S. farctum,* as shown by Berthold (1878)[134] was not found to occur in the culture that we obtained from Indiana University, Bloomington, culture collections (sic), but was composed of linear row of cells (Pl. 39, Fig. 6,7).[135] Thus, the same species may produce different types of prostrate system depending upon various environmental factors in addition probably to the nature of the substratum. Contrary to this, many species may have developed similar type (sic) of prostrate system.

In our opinion, a more reasonable explanation is available. The isolates of *S. farctum* used in this study and assembled from several sources *always* formed a disc, both in culture (in defined and undefined media) and when transplanted to the Blanco River, San Marcos, Texas.[136] Isolate S–5 (LB 439, Indiana University) *never* formed a disc-like basal system. The early germling stages, however, resembled those of *S. farctum,*[137] and there was some proliferation on the lateral

[132] S–5 is isolate LB 439, *S. farctum* Berthold, from the Culture Collection of Algae, Indiana University, Bloomington, Indiana. It was originally deposited by Butcher, the isolator, in the Cambridge Collection of Algae and Protozoa, The Botany School, Cambridge, England (Camb. B. 477/10b).

[133] LB 440, *S. farctum* Berthold, from the Indiana Culture Collection of Algae, Indiana University, Bloomington, Indiana. This organism was originally deposited by Reynolds, the isolator, in the Cambridge Collection of Algae and Protozoa, The Botany School, Cambridge, England (Camb. B 477/10a). The writers have had this isolate in culture throughout this investigation, although it was not as intensively studied as LB 439.

[134] Refer to Text-fig. 5, p. 76.

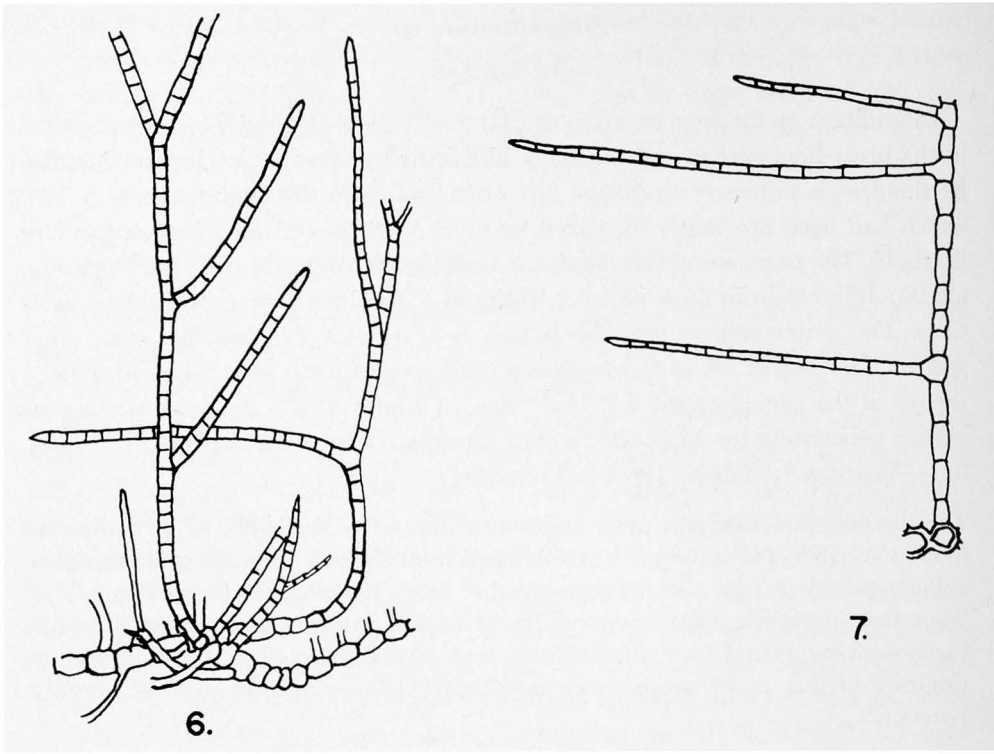
[135] Refer to Text-fig. 4, p. 64.

[a] Writers' isolate number S–5.

[b] Both LB 439 and LB 440 are listed as *S. farctum* Berthold by the Indiana University Culture Collection of Algae, Bloomington, Indiana.

[136] It is true, however, that a branching filament precedes the formation of a mature disc and, on occasions, the disc does not "fill in completely."

[137] Many other species also, for that matter.



Text-figure 4.—Camera lucida drawings by Islam (1963, Pl. 39, fig. 6, 7) of S. *farctum* Berthold (LB 439[a] and LB 440[b])

prostrate branches at the ends of the filamentous base in an undefined medium.[138] It is inconceivable to us that one and the same species can produce two such very different types of basal systems (as Islam implies).

Butcher (1932) emphasized the disc-like basal system of *S. farctum,* and isolate S–5 differs significantly in lacking it. The writers are at a loss, therefore, to understand why Butcher identified his isolate (S–5) as *S. farctum* Berthold.

The major differences between isolate S–5 and the four other isolates definitely associated by the writers with *S. farctum* Berthold are summarized in Table 8.

MORPHOLOGICAL OBSERVATIONS[139]—*Zoospores and germling stage*—Usually one, sometimes two, quadriflagellate zoospores were produced in each cell of the erect filaments and released through a small lateral pore in the cell wall (Fig. 253, 254). The zoospores *always* germinated to form an erect filament—type I (Fig. 249, 257).[140] From the lowermost cell of the upright filament, i.e., the zoospore (Fig. 249), an irregularly branching filament was subsequently produced.

Mature basal system—The mature basal system was a prostrate, branching fila-

[138] 3 BBMP: 1 SS.

[139] Morphological observations from cultural studies only.

[140] Refer to page 30.

TABLE 8. *Major differences between isolate S–5 (LB 439) and isolates of* S. farctum *Berthold (5–3C, 5–3F, 19–5–V, and 7–17)*

Basal System

Isolate S–5 never formed the complete, disc-like basal system which was so characteristic of the other isolates.

Zoospore Germination

Only type-I zoospore germination was observed in isolate S–5, whereas both type I and type II were seen in the other isolates.

Cell Length

The cells of the erect filaments of isolate S–5 were usually longer than those of the *S. farctum* isolates.

Branch Tips

In isolate S–5 multicellular, colorless hairs were the rule, rather than the exception.

Length of Maintenance

It was not possible to maintain a culture of isolate S–5 longer than 2–3 weeks in liquid culture, after which time the erect filaments released zoospores without induction and the culture became a mass of young germlings; *S. farctum* isolates could be maintained for 2–3 months in liquid culture.

Color Upon Aging

Four-month-old agar cultures of S–5 were bright orange. At the same age maintained under the same conditions, isolates of *S. farctum* were light-green to yellow-green.

ment of restricted or limited growth which produced short, prostrate lateral branches (Fig. 250, 252, 257, 258). Rhizoids were *very* rarely produced.

Erect system—Young, actively growing cells of the erect system were cylindrical and little constricted at the partition walls (Fig. 258). It was difficult to maintain this organism longer than 2–3 weeks in liquid culture, after which time the erect filaments either released zoospores or dissociated into single cells. At two months on $BBMPTB_{12}$ agar, the cells of the filaments became spherical and the cell walls thickened (Fig. 255, 256). The cells of the filaments often dissociated and the akinete-like cells were filled with starch (Fig. 256). However, no *Palmella* stage was ever found in this culture.

The width of the cells of the erect filaments was 5–6–10μ and the length (8–10)–15–35μ.

The types of branching usually found were alternate (Fig. 252, 258) or dichotomous (Fig. 257). Unbranched erect filaments (Fig. 257) were also common.

Long multicellular colorless hairs at the tips of the branches were the rule, not the exception (Fig. 253, 257). Multicellular colorless hairs were often produced

directly from the cells of the basal system (Fig. 249). Pointed or acute branch tips were also found (Fig. 258).

Colony characteristics—A 1-month-old culture on 1.5% $BBMPTB_{12}$ agar was matted or covered with distinct tufts (Fig. 259). Sometimes the *extreme* edges of the colony were composed of curved bundles of filaments.

Color upon aging—The color of the colony on 1.5% $BBMPTB_{12}$ agar in 100- × 13-mm culture tubes was as follows: 1-month—dark- to grass-green; 2 months—grass-green, slightly orange on top; 4 months—orange. This alga also discolored proteose agar at 2 weeks.

Descriptions of Stigeoclonium Species

As the writers have emphasized, specific determination in the genus *Stigeoclonium* in the literature is at present based on those very morphological attributes of the erect system which have been herein demonstrated to be extremely variable. A survey of the literature from Kützing (1843) to Islam (1963) reveals the difficulties encountered not only in identifying any living plant as a certain species, but also in correlating the descriptions of any one species as interpreted by several workers. In view of these facts, the writers had to choose between two alternatives in arriving at the specific disposition of the isolates studied in this investigation—i.e., either to describe each isolate or group of isolates as a new species, or to broaden the concept of some inadequately described species to encompass the variations demonstrated in this study. The former alternative was rejected because, in the writers' judgment, erecting new species would further confuse, rather than clarify, an already confused situation. They chose, instead, to take a recent critical work on the genus *Stigeoclonium* (Islam, 1963) as a foundation for the description of each species, and to modify such descriptions in accordance with data from our investigation.

Throughout the following discussion the letters in parentheses have been added to Islam's specific diagnoses to correspond to the points discussed below each such description.

Stigeoclonium helveticum Vischer

Islam (1963, p. 76) described *S. helveticum* as follows:

Thallus turf-like, cushion-forming (a), 1–5 cms. (b); filaments simple, long, thread-like, branches remote or sometimes several branches from adjacent cells; mostly alternate, rarely opposite or 2–3 from same point, developed by evection from any place of the cell (c); branches long, bluntly attenuating or ending in a colorless hair (d); cells of the main filament and branches cylindrical, at the septum usually not constricted or slightly so (e), 6–12.5μ in diameter, 1–8 times as long.

(a) The meaning of "thallus turf-like, cushion-forming" is somewhat obscure,

since Islam does not mention whether he is describing the thallus on agar or in a liquid medium.

(b) The length of the erect filaments of any *Stigeoclonium* plant is extremely variable depending on the age of the plant and the conditions of the culture; size can only be defined precisely when these factors are known; e.g., refer to p. 37 for a discussion of the length of the erect filaments of *Stigeoclonium*.

(c) This condition (i.e., branches developed by evection from any portion of the cell), though certainly found in our isolate of *S. helveticum* (Fig. 23), also occurred in all other *Stigeoclonium* isolates studied and cannot, therefore, be used to distinguish *S. helveticum* from other species. Islam notes (1963, p. 76):

> The branching habit is the only characteristic feature; sometimes the young branches appear like the branching habit of the blue-green alga *Scytonema*. Whether this is a growth-form in the culture medium or results from particular environmental conditions or whether it is a specific character must yet be determined.

(d) The terminal cell in young germlings (2–3 days old) often extended into a hyaline pointed tip. However, the tips of older filaments (over 1 week old) were always blunt under standard conditions;[141] see p. 44 regarding occurrence of hairs in *Stigeoclonium*.

(e) The shape of the cells of each of the isolates used in this investigation was found to be somewhat variable, depending on the age of the culture; see p. 38 for a discussion of cell shape.

To Islam's description of *S. helveticum* the writers would now add the following based on observations made during this investigation:

Basal system—Plants loosely attached to substrate by a sparse, rhizoid-like basal system developed from the lower or middle cells; in test-tube cultures,[142] plants not attached to the walls of the tube.

Erect system—Erect filaments of indeterminate length, unbranched or sparsely branched in a.g.[143] cultures. Filaments more branched in older.[144] cultures; the branches, if present, usually alternate, rarely approximate or opposite. Branch tips of a.g. cultures blunt under standard conditions. Cells of a.g. filaments cylindrical, becoming more constricted and thick-walled with age (2 months or more).

Colony characteristics[145]—Vermiform at 14 ×, margins composed of curved bundles of filaments.

[141] Standard conditions of culture are outlined on p. 12.

[142] Wherever "culture" is mentioned in the amplified descriptions which follow, standard conditions prevailed, unless otherwise specified.

[143] a.g. = actively growing cultures (2-week-old or younger).

[144] That is, more than 1 month old unless otherwise specified.

[145] Throughout the following discussion statements regarding colony characteristics are based on 1-month-old cultures in Petri dishes of 1.5% BBMPTB$_{12}$ agar.

Stigeoclonium aestivale (Hazen)
Colins (Emend.)

Islam (1963, p. 68) described *S. aestivale* as follows:

Plants in dense tufts on stones, aquatic plants, 2–5 (—15) mm long (a), light-green in color (b); erect filaments radiating from a palmelloid base (c) or creeping filaments composed of isodiametric cells, or from interwoven mass of narrow, downward-growing filaments and rhizoids, the latter may be profuse from basal cells (d); erect filaments dichotomously or alternately branched, simple, straight (e), branchlets few below, more above (f), erect, slender with attenuated tips, often ending in fine setae (g); cells thin-walled (h), slightly inflated and constricted (i), 7–12μ (rarely —15μ) in diameter, 2–6 times as long, above as long as broad and little more inflated (j).

(a) See (b) under *S. helveticum*, p. 67. The erect filaments attained a length of 50 mm in some of the writers' cultures of *S. aestivale*.

(b) Color is too much a factor of age of the plant and such environmental factors as light intensity and nitrogen concentration to be a valid specific attribute unless these conditions are cited.

(c) As used here, the meaning of the term "palmelloid" is somewhat obscure. Hazen (1902) did not define "palmelloid" in the original description of *S. aestivale*. It is doubtful that Islam means to imply here that the basal system is composed of single cells within a gelatinous matrix, as the term "palmelloid" indicates.

(d) Islam lists three different types of basal systems for this one plant. The writers' experience tends to refute such an occurrence. See p. 45 for a discussion of the reliability of the basal system as a specific attribute.

(e) Islam's use of "simple, straight" following a statement of "erect filaments dichotomously or alternately branched" is confusing. It is probable that the "simple, straight" erect filaments are just young filaments which may later branch.

(f) In the writers' experience, the exact placement of primary or secondary branches (i.e., "few below, more above") is more representative of individuals within a population than of the population as a whole. Refer to p. 40.

(g) Presumably "setae" here refers to "unicellular hairs." See p. 44 for a discussion of the confusing terminology regarding the nature of the branch tip in *Stigeoclonium*.

(h) See p. 39 for a discussion of cell-wall thickness in *Stigeoclonium*. Since the cell walls of all of the isolates in this investigation thickened with age and cells of all a.g. cultures had thin walls, this attribute, at least at the present, is of little value in specific determination.

(i) See (e) under *S. helveticum*, p. 67.

(j) The writers' measurements of cell width and length did not entirely correspond to those given by Islam for *S. aestivale*. The difficulty, however, in using this attribute for specific determination is discussed on p. 37.

Based on observations made during this investigation, the writers would now

emend Islam's description of *S. aestivale* as follows:

Basal system—Small filament from which prostrate lateral branches of restricted growth developed; prostrate lateral branches often rebranching and, sometimes, but not always, ending in colorless rhizoids.

Erect system—Erect filaments of indeterminate length, unbranched or sparsely branched in a.g. cultures; filaments more branched in 3–4-week-old cultures; the branches, if present, usually alternate, secund, or irregular. Downward-growing rhizoids often developed from cells of erect filaments. Cells of a.g. filaments cylindrical and little constricted at partition wall, becoming more spherical and thick-walled with age (2 months or more). Cells of main axis varied from 4–10μ in width and from 5–25μ in length. Branch tips in a.g. culture either blunt, acute, or with multicellular colorless hairs, the latter somewhat more common in older cultures than in a.g. cultures under standard conditions.

Colony characteristics—Vermiform or with few scattered erect or matted tufts at 14 ×, margins composed of curved bundles of filaments.

Stigeoclonium subsecundum (Kütz.) Kütz.

Islam (1963, p. 84) described *S. subsecundum*[146] as follows:

Plants very delicate (a), bright-green to yellowish-green in color (b); forming slimy (c) flakes or entangled with aquatic plants, on sticks, etc., mostly found in stagnant water (d); main filaments very sparsely branched below, rather long rhizoids develop from many cells above the base; branches more or less dichotomous or alternate, never opposite, often several branches developing from successive cells on the same side of the filaments, gracefully tapering to attenuated tips, rarely short-setiferous (e); some branches with the cells of the same character as the main axis, other branches may be small (f); cells of main axis long, cylindrical, with little or no constrictions at the partition wall (g); some cells of the main axis may be small and barrel-shaped above, followed by long, cylindrical cells (h); cells producing branches may be different from other cells; if so, they are slightly shorter and little inflated (i); cells of main filament 7–20μ in diameter, 3–10 (—12) times as long; chloroplast single, cylindrical, incomplete parietal plate (j).

(a) "Delicate" is an indefinite term; *S. subsecundum* is neither more nor less delicate than all of the other *Stigeoclonium* isolates examined by the writers.

(b) See (b) under *S. aestivale,* p. 68.

(c) If "slimy" here refers to a mucilaginous matrix surrounding the filaments, the matrix probably is a response of the plant to extremely unfavorable environmental conditions resulting from the depletion of nutrients in the medium or to

[146] Islam recognized two varieties of *S. subsecundum*, namely: *S. subsecundum* (Kütz.) Kütz. var. *subsecundum* and *S. subsecundum* var. *tenuis* (Nordt.) Islam. The latter variety, he said, "may be regarded as a young stage of the species."

other factors,[147] rather than an invariably present attribute. The writers' isolate of *S. subsecundum* did not form a mucilaginous matrix under standard conditions. See p. 39 for a discussion of the formation of mucilage in *Stigeoclonium*.

(d) Such an observation may be premature in view of the nature of Islam's work and the difficulty in identifying *Stigeoclonium* species in nature.

(e) The meaning of "short-setiferous" as used here is not clear. See (g) under *S. aestivale*, p. 68.

(f) This character was true of all of the *Stigeoclonium* isolates that the writers studied. In general, the size of the cells of the larger branches approached the size of the cells of the main axis; the cells of the younger branches were somewhat smaller. The general nature of this attribute renders it useless as a criterion for specific determination.

(g) See (e) under *S. helveticum*, p. 67.

(h) The writers found some differentiation of the cells of the main axis in this isolate, as well as in several other isolates studied in this investigation. See p. 38 for a discussion of cell shape as a taxonomic criterion.

(i) Since cell division is intercalary in the erect filaments of *Stigeoclonium*, it is not uncommon for branches to be produced from "slightly shorter and little inflated" cells. These are simply cells which have recently divided. See p. 34 for a discussion of the differentiation of branch-producing cells of the main filament in the genus *Stigeoclonium*.

(j) Such internal cellular organization is characteristic of the genus. Refer to p. 40 for a discussion of the internal structure of the cells of *Stigeoclonium*.

To Islam's description of *S. subsecundum*, the writers would now add the following based on observations made during this investigation:

Basal system—A small filament from which prostrate lateral branches of restricted growth developed. Numerous, slender, spreading rhizoids developed from the cells of the basal system, the latter sometimes appearing as a mass of rhizoids.

Erect system—Erect filaments extensive and of indeterminate length, unbranched or branched in a.g. cultures; the branches, if present, usually alternate, sometimes dichotomous or secund. Branches formed from the top or from the middle of the cells. Under standard conditions, branch tips in a.g. cultures blunt, sharp-pointed, or rarely with multicellular colorless hairs. Cells of a.g. erect filaments cylindrical, becoming *slightly* more constricted with age, but not forming thick-walled globose akinetes at 2 months on 1.5% BBMPTB$_{12}$ agar. Rhizoids developed from barrel-shaped or cylindrical cells of the erect filaments. Cells 4–10μ in width and up to 50μ in length.

Colony characteristics—Vermiform or with scattered erect or matted tufts at 14 ×, curved bundles of filaments at extreme edges.

[147] Islam (1963, p. 86) says: "In winter it (i.e., *S. subsecundum*) has been found under the ice in temperate areas, when the filaments are more mucilaginous."

Islam (1963, p. 86) said: "Sometimes the young stages . . . may look like *S. aestivale*." Again (1963, p. 68) referring to *S. aestivale*, Islam said:

Sometimes, this species (i.e., *S. aestivale*) comes closer to *S. subsecundum,* especially in younger forms. But, constant presence of small, slightly inflated cells, more setiferous branch tips, tufted and radiating filaments may be characteristics of this species and can be easily separated from *S. subsecundum.*

The writers acknowledge that their isolate of *S. subsecundum* (19–11–V) is closely related to the isolates of *S. aestivale* (8–3, Var 5, HP 4) used in this investigation. At the present time, however, they are of the opinion that the two species should be separated, not for the reasons enumerated above by Islam, but rather on the basis of the abundant production of rhizoids by isolate 19–11–V; otherwise, the two species appear to be very much alike.

Stigeoclonium tenue (Ag.) Kützing (Emend.)

Islam (1963, p. 102) states:

S. tenue is one of the most common and most polymorphic species of the genus, occurring in widely distributed areas. . . . The species may be perennial or annual and does not always show a "normal" healthy growth. . . . It shows various growth forms, some of them appearing entirely different from the typical species, and several varieties thus have been established by Kuetzing, Rabenhorst, Hansgirg, and others who failed to realize the range of variations of this species under different environmental conditions.

Islam himself recognizes two varieties, namely, *S. tenue* var. *tenue* and *S. tenue* var. *uniforme*. The two are distinguished by the simple branching habit of the former and the slightly greater cell size of the filaments of the latter which show the "tendency to produce long, plumose tufts at the tips of the branches and main filaments."

Islam (1963, p. 91) described *S. tenue* as follows:

Well-developed plants forming cushion, turf or tuft (a), lubricous, few mm. to 5–10 cms. (sometimes more) high (b); bright green (c); profuse erect filaments developed from prostrate parts (d); branches simple, alternate and opposite (e), usually developed from angular cells smaller than others (f); cells of main axis cylindrical, little constricted (g), 6–15 (rarely 18) μ in diameter, and 2–5(—6) times longer than broad [usually 10–12μ in diameter, and 2–5(—6) times longer] (h); branches gracefully attenuated or tapering into thin, colorless tips, rarely finely setiferous and usually without any long hairs (i); at the upper part the secondary branches may be short, scattered, or alternate, or long slender and crowded to form bushy, long-drawn tufts (j); chloroplast filling the smaller cells, in long cells occupying the middle portion (k); sometimes, prostrate part may be palmelloid or with profuse rhizoids (l).

(a) Here "cushion, turf or tuft" probably refers to the macroscopic appearance

of the plants in liquid rather than on agar. However, at best, the words are indefinite and contribute little to specific distinction.

(b) See (b) under *S. helveticum*, p. 67.

(c) See (b) under *S. aestivale*, p. 68.

(d) This is true of many species of the genus *Stigeoclonium* and, thus, is useless as a specific attribute.

(e) The writers did not find any isolate of *Stigeoclonium* in which the branching was equally "alternate and opposite." It is probable that Islam has here combined the descriptions of *S. tenue* given by earlier workers, some of whom described branching as alternate, while others described it as opposite.

(f) See (i) under *S. subsecundum*, p. 70.

(g) See (e) under *S. helveticum*, p. 67.

(h) In the writers' isolates of *S. tenue*, cells varied from 4 to 10μ in width and attained a length up to 30μ. Refer to p. 37 for a discussion of the difficulties involved in using cell width and length as a criterion for specific distinction.

(i) Islam's use of "rarely finely setiferous and usually without any long hairs" seems to be a contradiction in terminology. See (g) under *S. aestivale*, p. 68.

(j) See (f) under *S. aestivale*, p. 68.

(k) This is true of the genus *Stigeoclonium*. Refer to p. 40 for a discussion of the internal structure of the cells.

(l) See (c) under *S. aestivale*, p. 68, for a discussion of Islam's use of the term "palmelloid." Islam describes two types of basal systems for *S. tenue*. See p. 45 for discussion of the reliability of the basal system as a specific attribute.

Based on observations made during this investigation, the writers would now emend Islam's description of *S. tenue* as follows:

Basal system—Under standard conditions, an extensive, prostrate filament of indeterminate (unlimited) growth from which lateral branches arise in an irregular manner, rebranching, and becoming as extensive as the first filament. Central cells globular or barrel-shaped. Ends of lateral prostrate filaments usually extend into colorless, "corkscrew"-like rhizoids which may be quite long.

Erect system—A distinct, extensively developed, erect system from cells of basal system; erect filaments of indeterminate length; unbranched or sparsely branched in a.g. cultures; filaments more branched in 3–4-week-old cultures; the branches, if present, usually alternate, often secund, dichotomous, or pseudodichotomous. Downward-growing rhizoids sometimes develop from cells of erect filaments, but are never profuse. Cells of a.g. filaments cylindrical and little constricted at partition wall, becoming more constricted with age (2–3 weeks old), and subsequently forming spherical, thick-walled cells (2 months or more). These akinete-like cells often dissociate into single cells without formation of any mucilage. Cells of main axis varied from 4 to 10μ in width and from 5 to 30μ in length. Under

standard conditions branch tips in a.g. cultures either blunt, sharp-pointed, or with multicellular colorless hairs, the latter sometimes quite long.

Colony characteristics—Caespitose or matted at 14×.

Stigeoclonium pascheri Vischer comb. nov.

Vischer erected the genus *Caespitella* in 1933. He distinguished it from *Stigeoclonium* largely on the basis that cell division in *Stigeoclonium* was intercalary, while that of *Caespitella* was entirely apical.[148] Vischer was at the time intensively studying both *S. helveticum* and *C. pascheri*. These two organisms differ greatly in growth pattern both in liquid and on agar (cf. Fig. 31 and Fig. 168). In our opinion, this divergency may have influenced Vischer in his belief that the organisms belonged to different genera. However, intermediate forms exist which make the difference less significant than Vischer supposed. Accordingly, the writers propose the abandonment of the genus *Caespitella* and transfer of *C. pascheri* to the genus *Stigeoclonium* as *S. pascheri* Vischer comb. nov.

Based on observations made during this investigation, the writers would now add the following to Vischer's description:

Basal system—Under standard conditions, an extensive prostrate filament of indeterminate (unlimited) growth from which lateral branches developed in an irregular manner, rebranching, and often becoming as extensive as the first filament. Central cells globular or barrel-shaped. Rhizoids seldom produced from terminal cells of the prostrate filaments.

Erect system—Erect filaments of variable length; may be very long in unaerated cultures, or in aerated cultures not well developed and inconspicuous, the latter easily confused with the upward proliferations of detached basal filaments in 3-week-old or older cultures. Aerated cultures form discrete colonies on glass substrates. A.g. cultures unbranched or sparsely branched, more branched in 2–3-week-old cultures; the branches, if present, usually alternate or secund. Cells of a.g. filaments cylindrical and little constricted at partition wall, becoming more barrel-shaped with age (3 weeks–1 month); subsequently forming spherical, thick-walled akinete-like cells (2 months or more). Cells of main axis varied from 4 to 10μ in width and from 10 to 36μ in length. Branch tips either blunt or slightly pointed in a.g. cultures under standard conditions.

Colony characteristics—Caespitose or matted at 14×.

Stigeoclonium variabile (Nägeli) Islam

Islam (1963, p. 56) said *S. variabile* "is one of the most extremely [sic] poly-

[148] Intercalary cell division does occur in the erect filaments of the genus *Stigeoclonium*. However, cell division in the prostrate filaments is apical. Refer to footnote, p. 37. The writers found intercalary cell division in the erect filaments of *S. pascheri* also (Fig. 149).

morphic *Stigeoclonium* species, the true nature of which must be determined by cultural and comparative studies." Table 3 of this report reveals the numerous "species" which Islam considered to be growth forms of *S. variabile.*

Islam (1963, p. 58) emended the description of *S. variabile* as follows:

Plants bright green (a), usually small (b), sparsely-branched, mostly alternate, at the base somewhat dichotomously branched, very rarely opposite, sometimes profusely branched (especially in culture) (c); branches may be long and slender or short and spine-like, branch apex usually sharply pointed or acute, rarely slightly setiferous, but without long terminal hair (d); thallus developing pseudoparenchymatous or monostromatic prostrate part of round or angular cells, about 15–20μ in diameter (e), cells of erect filaments rectangular (f), cylindrical, or slightly inflated (g), 6–11μ in diameter, usually 1–2 times (rarely 3 times) as long (in the cells of the branches, especially near the tip somewhat longer); cell-wall usually thin but in unfavorable conditions may be greatly thickened (h); cell may divide diagonally or longitudinally in old filaments to produce swarmers (i).

(a) See (b) under *S. aestivale,* p. 68.

(b) See (b) under *S. helveticum,* p. 67.

(c) Erect filaments of *S. variabile* were observed by the writers to be unbranched, sparsely branched, or profusely branched depending on the age of the culture. Dichotomous branching was seldom observed.

(d) "Rarely slightly setiferous, but without long terminal hair" is a contradiction in terminology. See (g) under *S. aestivale,* p. 68.

(e) Two types of basal systems are mentioned here. See p. 45 for a discussion of the reliability of the basal system as a taxonomic criterion.

(f) "Rectangular" describes two-dimensional objects, not three-dimensional ones such as cells.

(g) See (e) under *S. helveticum,* p. 67.

(h) The cells of all of the isolates studied in this investigation were thin-walled in a.g. cultures, the walls becoming thicker as the culture aged. Thick-walled akinetes were commonly found in 2-month-old cultures.

(i) Prior to zoospore formation, the cells of several of the isolates studied by the writers divided diagonally. Godward (1942) described longitudinal division prior to zoospore formation in *S. amoenum.* This character is, therefore, true of the genus *Stigeoclonium* and is of little value for specific determination.

To Islam's description of *S. variabile* the writers would now add the following based on observations made during this investigation:

Basal system—Branching filament with a predominant main filament from which prostrate lateral filaments of limited growth developed, the latter rebranching to form a very compact basal system—almost a disc—of spherical, akinete-like cells.

Erect system—Erect filaments of indeterminate and variable length; unbranched or sparsely branched in a.g. cultures, profusely branched in older cultures (3

weeks–1 month); branches, if present, usually alternate, often secund, irregular, approximate, rarely dichotomous or opposite. Cells of a.g. filaments cylindrical and little constricted at partition wall, becoming more spherical with age (3 weeks–1 month), and subsequently thick-walled (2 months or more). Branch tips of a.g. culture blunt, very pointed or acute, or with long multicellular colorless hairs.

Colony characteristics—*Schizothrix*-like tufts at 14×, occasionally matted at center or with bundles of curved filaments at extreme edge.

Stigeoclonium farctum Berthold

Because of the writers' particular interest in this species, a rather detailed discussion of the history of *S. farctum* is presented below.

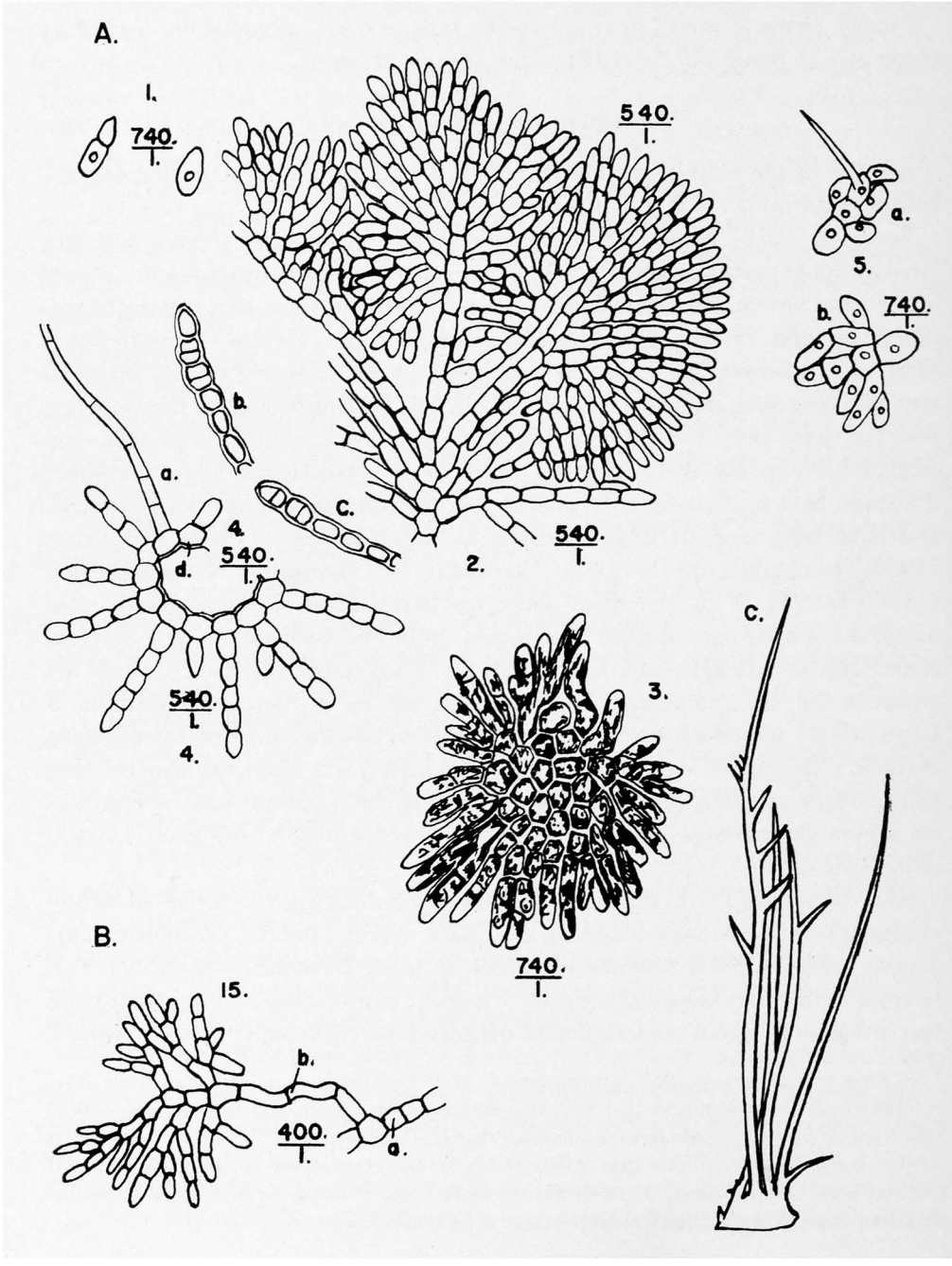
Berthold (1878) reported and illustrated a species of *Stigeoclonium* having a large pseudoparenchymatous, disc-like basal system. Although the distinctive nature of the basal system first attracted his attention, Berthold chose to name the organism, *S. farctum*, "wegen der dichtgedrängten aufrechten Zweige." He stated that short, erect filaments (4–7 cells in length) developed from the cells of the basal system. These erect filaments, he said, occasionally branched; most of the branches, however, were only 1–2 cells in length. Originating from the basal cells, and located between the erect filaments, were long, multicellular hairs. Berthold's drawings, here in Text-fig. 5, represent the classical illustrations for this species, and have been used by many authors, including Heering (1914) and Printz (1964), to characterize the species. Berthold's only illustration of the erect filaments (Text-fig. 5–4), described above, is characteristic of young germlings of many, if not all, *Stigeoclonium* species and cannot be associated with *S. farctum* alone. The writers have seen many such germlings, especially those that do not attach to the substratum and develop on the surface of the culture medium.[149] Islam (1963) also noted several similar illustrations for other *Stigeoclonium* species. Berthold's description of "occasionally branched" erect filaments and colorless hairs originating from the cells of the pseudoparenchymatous basal system does not accurately represent the *only* expression of the morphology of the erect system of this species.[150]

After Berthold (1878) described and illustrated this epiphytic species of *Stigeoclonium*, several workers observed organisms having similar pseudoparenchymatous, disc-like basal systems, yet differing from Berthold's description of *S. farctum* in the morphology of the erect system. It is interesting to note at this point that although Berthold's description of the pseudoparenchymatous basal system of

[149] Refer to p. 48 for a possible explanation.

[150] Berthold also mentioned that the erect filaments lengthened and branched in culture (exact conditions not stated) in such a manner that *S. farctum* could not be distinguished from another species, namely, *S. lubricum*. Almost every filament terminated in a long, multicellular colorless hair. Unfortunately, Berthold did not include any drawings of these branching erect filaments, and this observation was largely ignored by later workers.

S. farctum was not changed by later workers, the description of the erect filaments was supplemented and modified in many ways as indicated by the italicized attributes in Table 9. Such organisms were usually treated as distinct entities; i.e., new species or varieties. Since nearly all of these reported variations in the erect



system can be demonstrated in the same organism in culture, it seemed evident to us that the description of the erect system of *S. farctum* must be broad enough to encompass them.

That the circumscription of *S. farctum* is yet incompletely understood is illustrated by the statement from Islam (1963, p. 53): "This species is highly variable in habit. The exact nature of this species is still to be determined." In our opinion, the confusion with regard to the characterization of this very distinctive *Stigeoclonium* species is the result of random observations, in different places and under varying conditions. Such observations make it impossible to assert that morphological traits observed in one organism may also be assigned to a very similar organism in which they have not been actually seen. Continuous observation, under controlled conditions, provides the best, and perhaps the only, method of fitting isolated "frames" into a "whole picture."

Islam (1963, p. 53) described *S. farctum* as follows:

Thallus epiphytic or endophytic (sometimes free-floating) (a) forming a cushion-like prostrate part from which erect filaments develop (b); cells of prostrate thallus more or less angular, compact, nearly isodiametric, forming a pseudoparenchymatous or monostromatic base (c), almost every cell producing an erect filament, unbranched for considerable distance, then alternately branched (d); cells of erect filaments cylindrical, may be slightly inflated (e); branch tips usually blunt, rarely ending in a multicellular colorless hair; cells of main filament 6–7μ–(8μ or little more) in diameter, 1–2 times as long, seldom more, especially at the branch tip (in culture medium cells may be longer) (f).

(a) Such a broad description of habitat as "epiphytic or endophytic (sometimes free-floating)" is of little value for specific determination. The writers doubt that *S. farctum* is endophytic.

TEXT-FIGURE 5. *Stigeoclonium farctum* Berthold[a]

[a] Berthold (1878) *Untersuchungen über die Verzweigung einiger Süsswasseralgen.* Explanations for figures are directly from the German.

A. Tafel 2

Fig. 1, 5. Germling 5(a) already with upright, hair-forming, sharp-pointed part.

Fig. 2. Part of an older basal system initially wtih less, later with very thick ramification.

Fig. 3. Young basal system with similar beginnings of very profuse branching, showing the cell contents (from culture).

Fig. 4. Creeping filament (d) with short erect filaments and a hair (a). (b), (c) erect filaments, in process of forming zoospores.

B. Tafel 1

Fig. 15. Part of a young plant of *S. farctum*

(a) an erect filament of the young plant

(b) a "Wurzelfaden" resulting from a cell of the filament, the fourth cell of which has found a firm substratum and from which the usual basal system is produced.

***Stigeoclonium farctum* var. *rivulare* Butcher**

C. Illustration from Butcher (1932) of the erect system of *S. farctum* var. *rivulare*.

TABLE 9. *Observations of organisms similar to* S. farctum *Berthold*[a]

1. Möbius (1888) described a species of *Stigeoclonium* having a large, disc-like basal system and *short, erect filaments, 2–3 cells in length, which ended in multicellular colorless hairs.*

2. Huber (1892) noted that *hairs sometimes developed in place of erect filaments* in epiphytic species of *Stigeoclonium* with well-developed basal systems.

3. Hansgirg (1893) described *S. farctum* var. *pygmaeum*[b] in which the *erect filaments, terminating in hairs, branched immediately above the disc-like base.*

4. Fritsch (1903) established *S. farctum* var. *simplex* in which *erect filaments were unbranched for a considerable distance above the disc-like base.* Fritsch found that the branches ended in terminal hairs more often in laboratory cultures than in collections taken directly from nature.

5. Butcher (1932) described *S. farctum* var. *rivulare,* obtained from a calcareous stream in England, in which *the erect system, more extensively developed than that described by Berthold, seemed to lack terminal colorless hairs in nature.* Concerning the morphology of the erect filaments, Butcher (p. 297) said: "The apex of an erect filament is first blunt, but later acute, acuminate, or sometimes terminated by a hair. The filaments are very narrow (4μ in diameter), sparsely branched, with short and alternate branches. The cells are very variable in length, being either isodiametric or elongated . . . hairs are very rarely produced and have been seen more frequently in samples left growing under laboratory conditions than in plants taken directly from the river."[c]

[a] Italicized attributes represent departures from Berthold's description of the erect system of *S. farctum.*

[b] Although Hansgirg himself subsequently changed *S. pygmaeum* Hansgirg to *S. farctum* var. *pygmaeum,* Islam (1963) considered both *S. pygmaeum* Hansgirg and *S. farctum* var. *pygmaeum* Hansgirg as synonyms of *S. variabile* Nägeli. Islam's illustrations (Pl. 40, Figs. 1–5) of *S. variabile* Nägeli are drawn from Hansgirg's type specimen of *S. pygmaeum.* The writers suggest that this association confuses the understanding of both *S. variabile* and *S. farctum.*

[c] Butcher's illustration of the erect system of *S. farctum* var. *rivulare* is reproduced in Text-fig. 5, p. 76.

(b) Islam's use of "cushion-like" is not clearly understood. In *Stigeoclonium* erect filaments generally develop from the "prostrate part."

(c) Two types of basal systems are mentioned here. See p. 45 for a discussion of the reliability of the basal system as a specific attribute, and p. 63 for a discussion of Islam's reasons for including a "monostromatic base" in the description of *S. farctum.*

(d) See (f) under *S. aestivale,* p. 68.

(e) See (e) under *S. helveticum,* p. 66.

(f) Conditions of culture need to be included. The writers' measurements of cell width and length (under standard conditions in culture) approximated those given by Islam.

To Islam's description of *S. farctum* the writers would now add the following based on observations made during this investigation:

Basal system—Short, prostrate filament of restricted growth from which prostrate lateral filaments developed in an irregular manner, rebranching, and becoming as long as the main filament. Lateral prostrate filaments developed adjacent to each other, the whole forming a pseudoparenchymatous, *Coleochaete*-like disc.

Erect system—Erect filaments profuse from basal cells; extensive or less so; unbranched or sparsely branched in a.g. cultures; more branched in 3–4-week-old cultures; branches, if present, usually alternate, dichotomous, secund, irregular, and approximate. Downward-growing rhizoids often profuse from older (3 weeks or more) cells. Cells of a.g. filaments cylindrical and little constricted at partition wall, becoming more barrel-shaped with age (3 weeks–1 month), and subsequently forming thick-walled, spherical, akinete-like cells (2 months or more). Branch tips in a.g. cultures blunt, pointed, or with multicellular colorless hairs, the latter somewhat more common in older cultures than in a.g. cultures under standard conditions. Erect system often reduced to multicellular colorless hairs in a.g. cultures in organically enriched medium (3 BBMP: 1 SS).

Colony characteristics—Distinct *Schizothrix*-like tufts at 14×.

Conclusions

During this investigation the writers examined approximately 100 unialgal isolates of *Stigeoclonium*. Axenic cultures were obtained of 60 of these and after a careful preliminary study, 20 cultures which represented diverse characteristics of the group were chosen for intensive study and investigation. These 20 isolates were observed under standard cultural conditions in a defined medium, as well as in an undefined medium (i.e., supplemented with soil supernatant). In addition, certain of the isolates were transplanted to a natural habitat in order to correlate morphological observations of laboratory cultures with those grown "in the field."

The primary objectives of this study were two-fold: (1) to evaluate the taxonomic criteria currently used to delimit species in the genus *Stigeoclonium*; and (2) to determine whether *Caespitella* Vischer should be maintained as a separate genus or combined with *Stigeoclonium*.

If one relies on morphological attributes for delimiting species, the attributes chosen should be the least variable of those which comprise the organism. Unfortunately for the collector who wishes to identify to the specific level every alga in a natural collection, a reliable attribute is not necessarily an obvious one. Such is the situation in the algal genus *Stigeoclonium*. The most obvious morphological attributes—those of the erect system of the thallus (e.g., size and shape of the cells, differentiation of the cells of the main axis, branching habit, nature of the branch tip, etc.)—have been shown by the writers and by certain earlier investigators to be extremely variable and hence unreliable. Intensive study in controlled cultural

conditions has shown that variations exist in the erect filaments when environment cannot be a contributing factor to such variation. Plants and animals do not exist as "species" in nature (or in culture) but rather as individuals. Some variation among such individuals within a group is to be expected, particularly in sexually reproducing organisms. A completely unrealistic number of species results when an investigator seeks to describe each variation encountered as representative of a different species.

In our opinion, the validity of any species of *Stigeoclonium* which has been noted from one or two isolated collections and which is described only in terms of the above-mentioned attributes of the erect system—and the literature contains many such "species"—must be suspect; in fact, the writers would completely disregard such species.

Furthermore, a thorough search of the literature finally convinced us that successively later taxonomists have in many cases so modified the descriptions of a given species as presented by their predecessors that they have become unrecognizable. This in most instances has been done without adequate evidence.

The writers found the morphology of the basal system to be a more consistently reliable attribute than the characteristics of the erect system. Accordingly, the isolates studied in this investigation were grouped on the basis of the morphology of the basal system. The 20 cultures which were studied intensively manifested three main types of basal systems; however, sub-categories could be distinguished in several of the main types. Such a grouping will inevitably lead to fewer "species" of *Stigeoclonium* than would be described from attributes of the erect system above.

Although the writers realize that emphasis on a characteristic not readily available in natural collections will not facilitate specific identification of *Stigeoclonium* in the field, convenience cannot be the basis for sound phylogenetic taxonomy.

Sixteen of the 20 isolates studied in this investigation have been assigned by us to six previously described species of *Stigeoclonium*, inasmuch as those species, although somewhat inadequately described, did not specifically exclude the organisms in question. The descriptions of four of these six species were amplified to encompass the variations encountered in this investigation; these amplified species comprise *S. helveticum* Vischer, *S. subsecundum* (Kütz.) Kützing, *S. variabile* (Nägeli) Islam, and *S. farctum* Berthold. The writers should mention that *S. helveticum* Vischer exhibited many of the characteristics of the genus *Ulothrix*. Future study may reveal that *S. helveticum* should be removed from the genus *Stigeoclonium*. Isolate S–5 (LB 439, *S. farctum* Berthold, Indiana University, Bloomington, Indiana) is not *S. farctum*. For the present, the writers withhold judgment as to the specific disposition of this organism. Descriptions of *S. tenue* (Ag.) Kützing and *S. aestivale* (Hazen) Collins were emended by the writers.

In addition to morphological attributes of the basal system, it was found that colony characteristics on agar were a reliable and most useful specific characteristic.

Certain other aspects of the genus *Stigeoclonium* were intensively studied although they had no particular taxonomic significance. Specifically, the writers thoroughly reviewed the literature regarding reproduction in *Stigeoclonium*, a study which revealed an amazing lack of thoroughly convincing reports. It also became clear that the *Palmella* stage (*sensu* Cienkowski, 1876) did not occur under the conditions of this investigation, or from increasing the osmotic pressure of the solution as reported in the literature. The writers prefer the term akinete for the so-called *Palmella* stages.

The second objective, as noted above, was to appraise the validity of the genus *Caespitella* Vischer. In the writers' opinion, no valid reason exists for separating *Caespitella* from *Stigeoclonium*, and, accordingly, the three *Caespitella*-like isolates (seemingly identical) in this study have been removed to the genus *Stigeoclonium* as *S. pascheri* Vischer comb. nov.

Finally, although a number of additional attributes connected with the sexual process (zygote form, wall thickening, gamete organization, etc.) would broaden and strengthen the system of classification in the genus *Stigeoclonium*, the conflicting accounts of the sexual process now in the literature and its sporadic and vicarious occurrence (along with our present inability to evoke it at will in laboratory culture) all force us to rely at present on such a system as herein presented.

Cultures of the following isolates have been deposited in the Culture Collection of Algae, Indiana University:

S. aestivale, 8–3
S. farctum, 19–5–V and 5–3C
S. pascheri, 18–3
S. subsecundum, 19–11–V
S. tenue, Var 1
S. variabile, Jo

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FIGURES 1–8

Effect of aeration on growth of *Stigeoclonium* sp.

Aerated[a] and unaerated 1-month-old cultures in $BBMPTB_{12}$. Note the more luxuriant growth, especially of the erect filaments, in the aerated flasks; and the tendency of the algae to grow at or on the surface of the culture medium in unaerated flasks.

Fig. 1. *S. subsecundum* (19–11–V)

Fig. 2. *S. pascheri* (10–2)[b]

Fig. 3. *S. variabile*

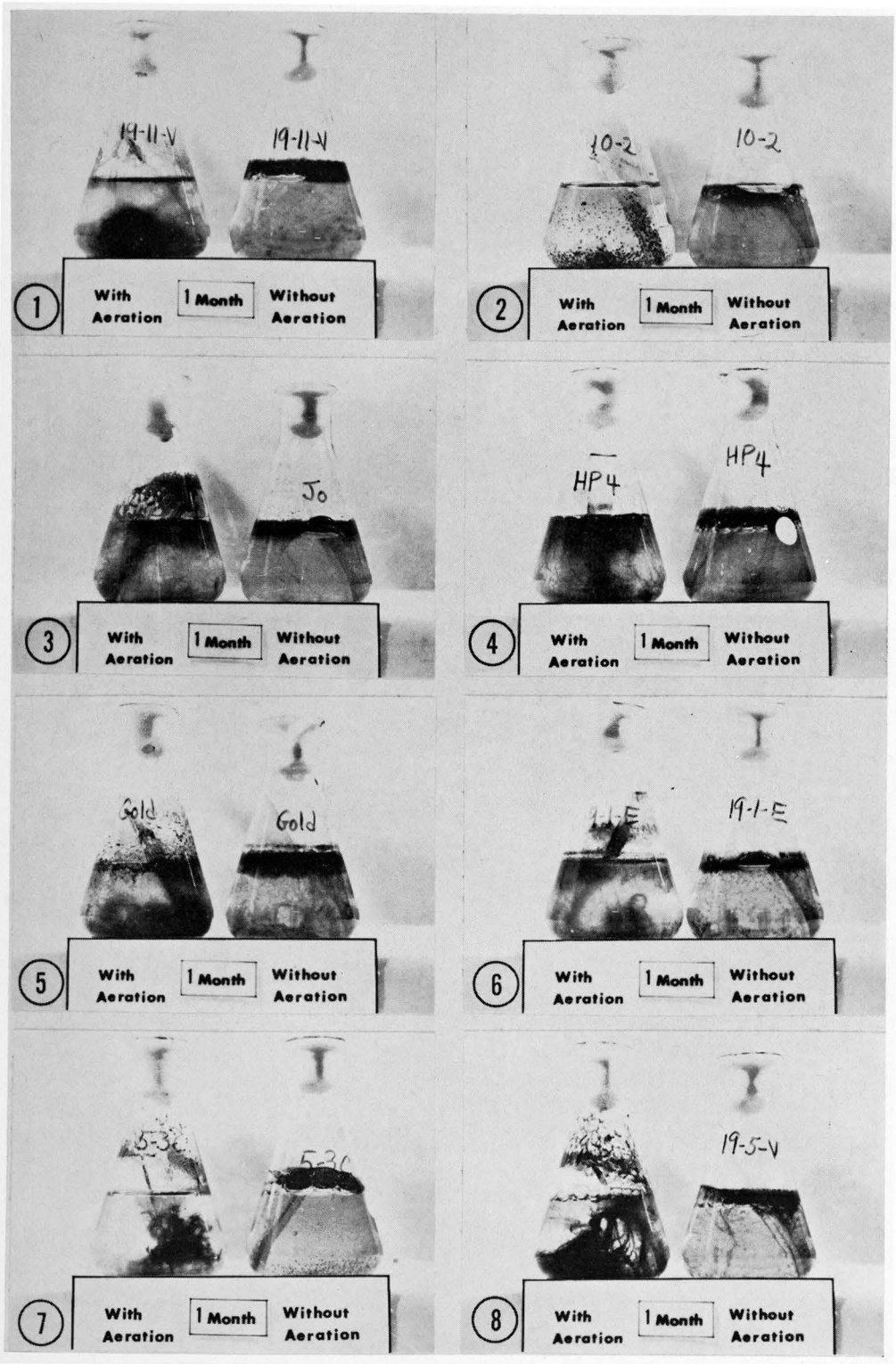
Fig. 4. *S. aestivale* (HP 4)

Fig. 5, 6. *S. tenue* (Gold, 19–1–E)

Fig. 7, 8. *S. farctum* (5–3C, 19–5–V)

[a] Aerated with 2–5 % CO_2 in air.

[b] A similar change in gross morphology was seen in isolates 18–3 and Ca 421. The close relationship of these three organisms is discussed later.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19-11-V
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19-5-V
7 With Aeration 1 Month Without Aeration
8 With Aeration 1 Month Without Aeration

FIGURES 9–13

Growing *Stigeoclonium* in Blanco River

Fig. 9, 10. Wire-enclosed wooden boxes used to grow laboratory cultures in natural habitat.

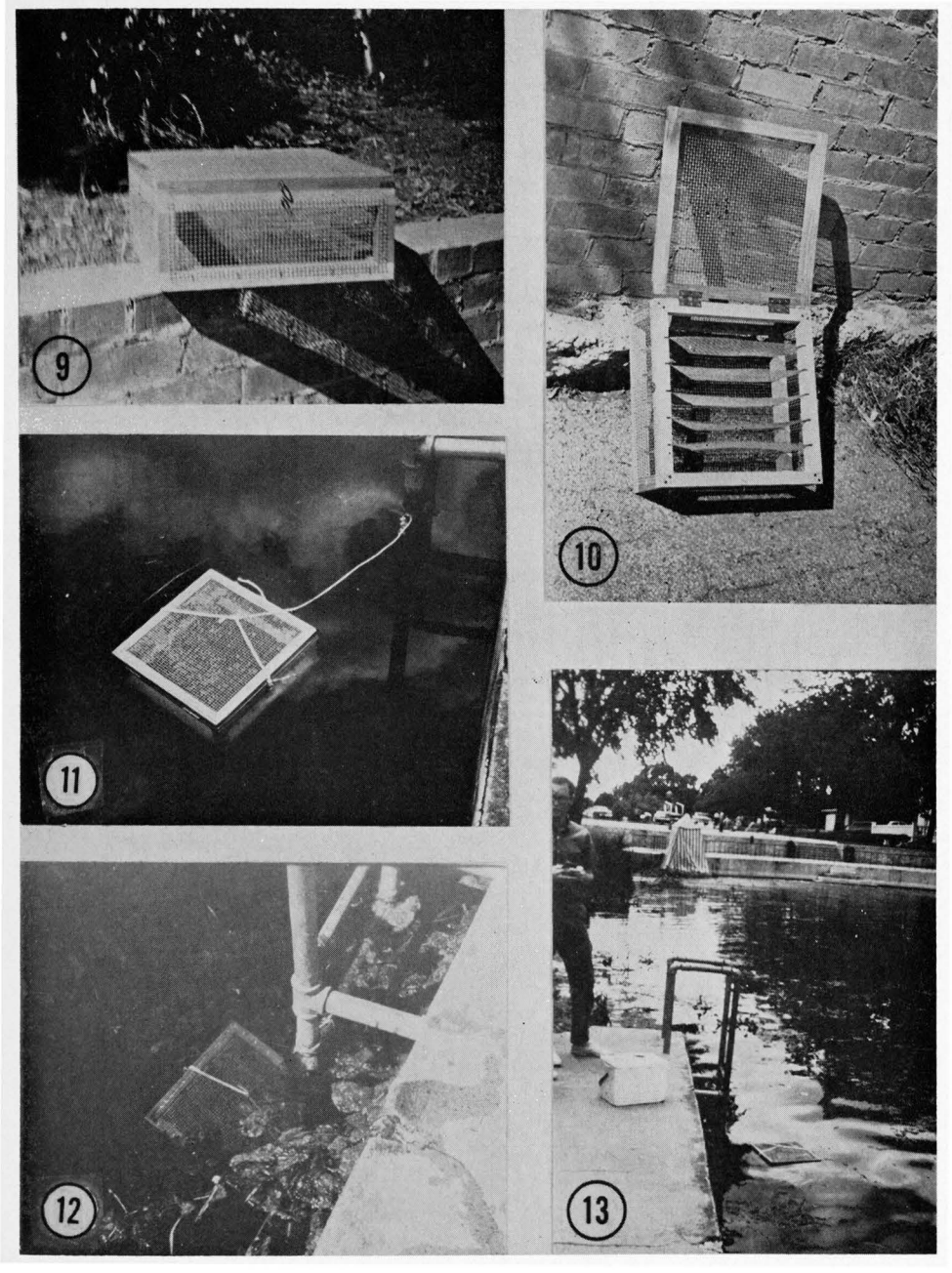
Fig. 9. View of box.

Fig. 10. Wire racks to which germling-covered slides were secured. Six slides were attached to each rack; 10 organisms could be grown at one time.

Fig. 11. Immediately after placing box in the Blanco River, San Marcos, Texas.

Fig. 12. Two weeks later, at time of collection.

Fig. 13. View of Blanco River at the Aquarena Park in San Marcos, Texas.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FIGURES 14–24

***Stigeoclonium helveticum* Vischer**
(Size scale in microns)

Fig. 14–17, 20. Variation of cell width and length.

Fig. 14, 15. Recent intercalary division (arrow).

Fig. 16. Extremely long cell; band-shaped chloroplast occupying center portion of cell.

Fig. 18, 19. Rounded, constricted cells from 4-week-old inoculum.

Fig. 19. Thin transverse wall probably indicating division of cell contents prior to zoospore formation, or to branch formation (arrows).

Fig. 21. Nature of branch tip (t).

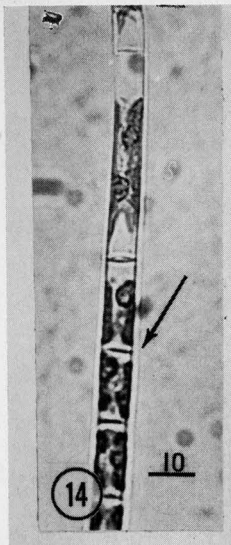
Fig. 22–24. Branch formation.

Fig. 22, 23. Enation of cell contents (arrow).

Fig. 24. Formation of transverse wall and subsequent evection to form alternate branching pattern (arrow).

Conditions of culture

Fig. 14-24. Isolate S–4; $BBMPTB_{12}$ aerated with 2–5 % CO_2 in air; 1–2 weeks after inoculation.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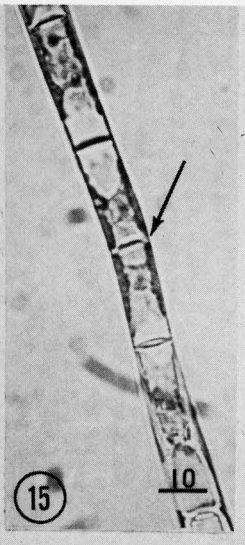
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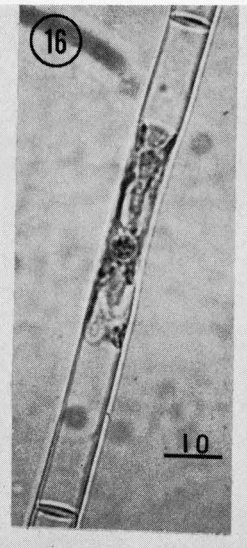
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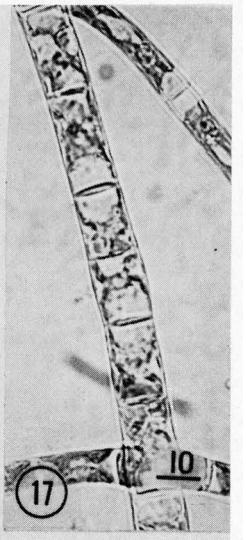
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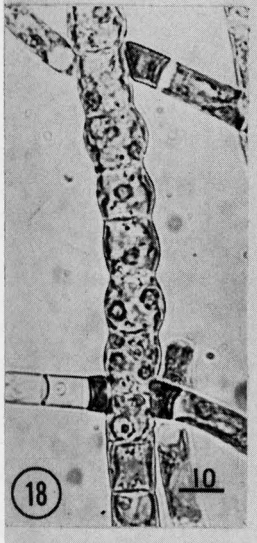
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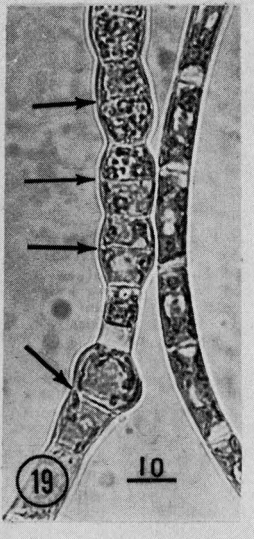
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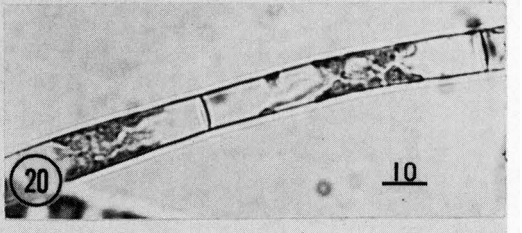
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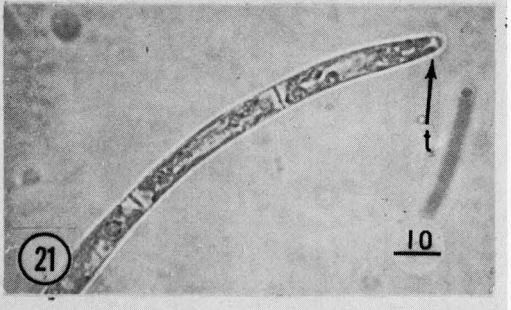
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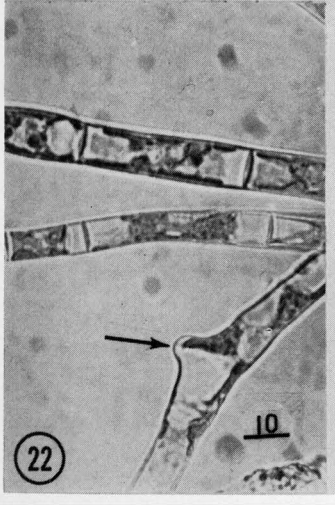
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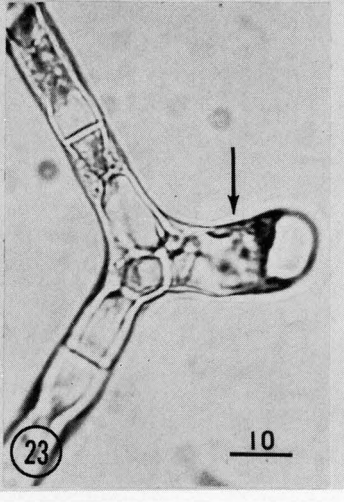
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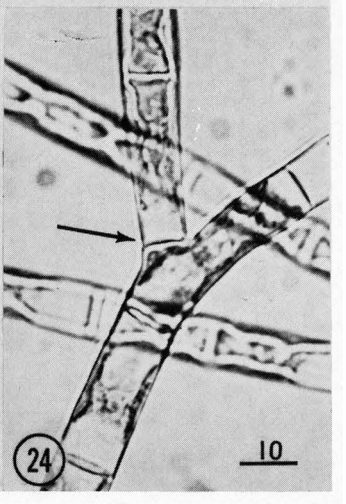
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FIGURES 25–29

Stigeoclonium helveticum **Vischer**
(Size scale in microns)

Fig. 25. Profuse branching from spherical cells of 4-week-old inoculum (2 weeks after transfer to fresh medium).

Fig. 26. Rhizoids (r) that attached erect filaments to substrate.

Fig. 27. Thick-walled (w), akinete-like cells.

Fig. 28, 29. Actively growing, sparsely branched, erect filaments.

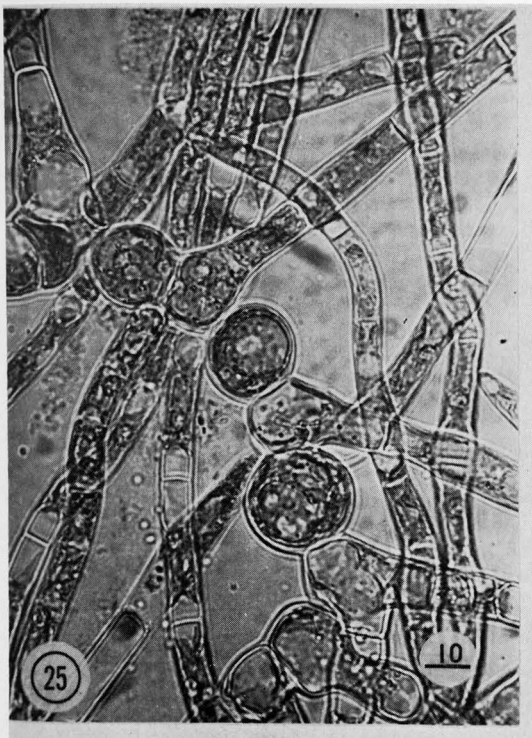
Fig. 28. Formation of erect filaments from fragments of 4-week-old inoculum (2 weeks after transfer to fresh medium).

Fig. 29. Early branch formation (arrow).

Conditions of culture

Fig. 25,26, 28, 29. Isolate S–4; BBMPTB$_{12}$ aerated with 2–5 % CO_2 in air; 1–2 weeks after inoculation.

Fig. 27. Isolate S–4; 1.5 % BBMPTB$_{12}$ agar; 2 months after inoculation.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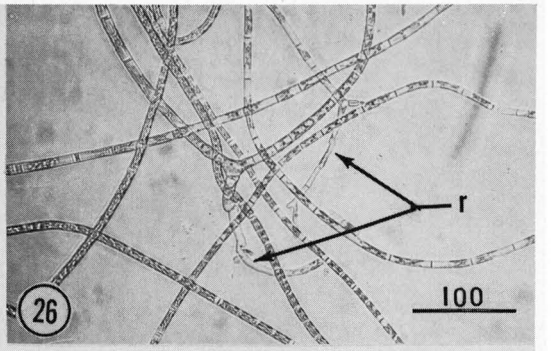
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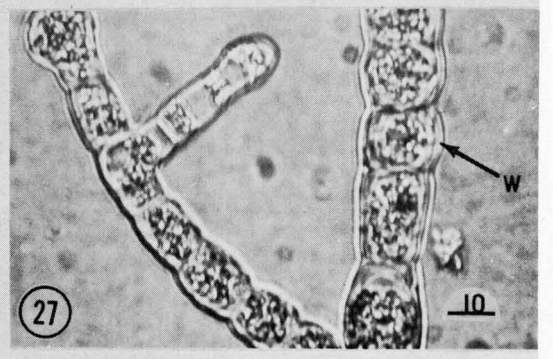
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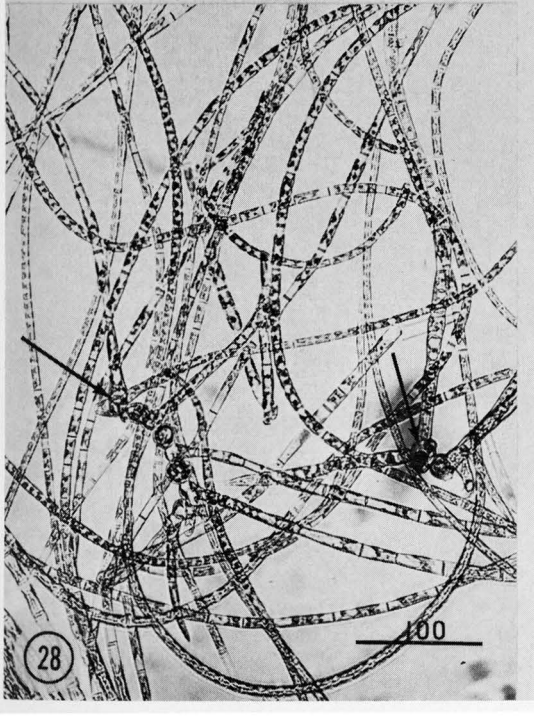
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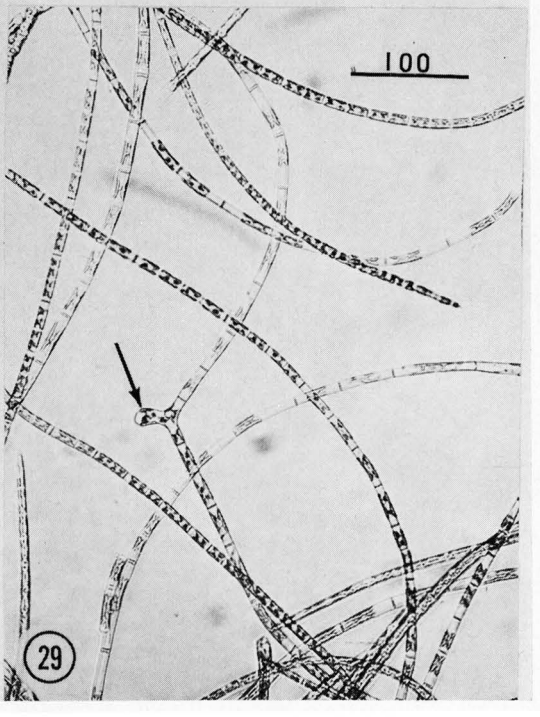
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FIGURES 30–31

***Stigeoclonium helveticum* Vischer**

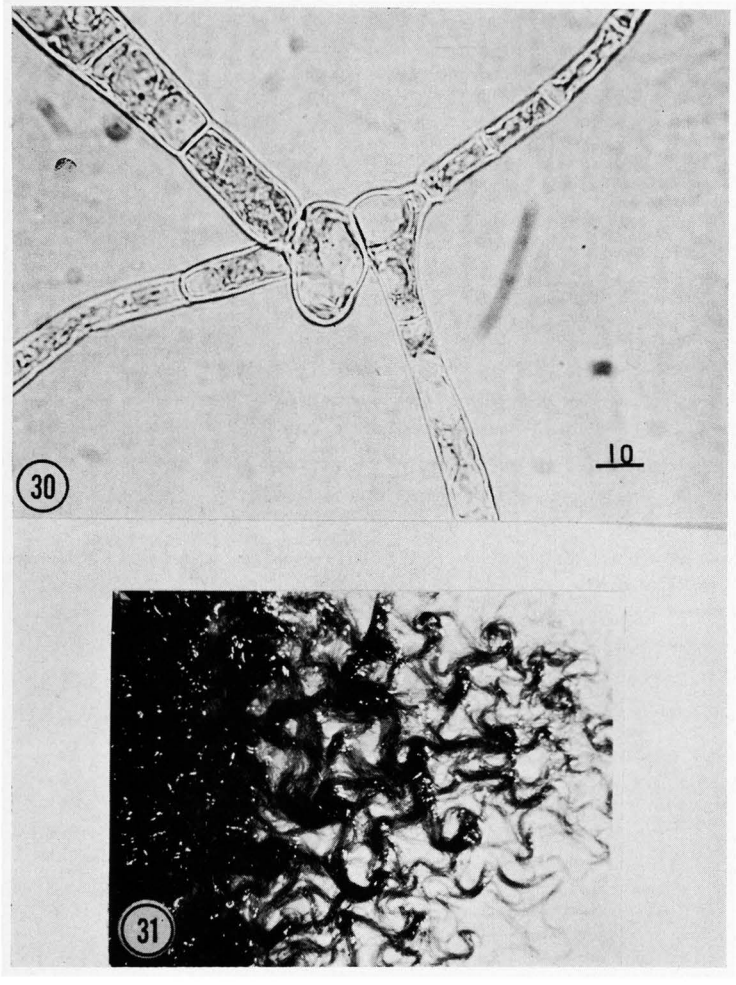
Fig. 30. Formation of approximate branches (size scale in microns).

Fig. 31. Flat, vermiform colony; edges composed of curved bundles of filaments (× 24).

Conditions of culture

Fig. 30. Isolate S–4; $BBMPTB_{12}$ aerated with 2–5 % CO_2 in air; 2 weeks after inoculation.

Fig. 31. Isolate S–4; 1.5 % $BBMPTB_{12}$ agar; 1 month after inoculation.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FIGURES 32–43

***Stigeoclonium aestivale* (Hazen) Collins (Emend.)**
(Size scale in microns)

Fig. 32, 35. Development of erect filament (e) from zoospore; unilateral formation of first prostrate filament (1).

Fig. 33, 36. Development of erect filament (e) from zoospore; unilateral formation of first prostrate filaments; beginning of bipolar germination (arrow).

Fig. 34, 37. Bipolar germination of zoospore; site of development of erect filament (e) from zoospore.

Fig. 38–42. Young plants showing development of erect filaments (e) from cells of prostrate thallus and original zoospore (z).

Fig. 43. Small branching prostrate system; long, alternately branched (b) erect filaments terminating in multicellular colorless hair (h).

Conditions of culture

Fig. 32–34. Isolate 8–3; $BBMPTB_{12}$; 2 days after inoculation.

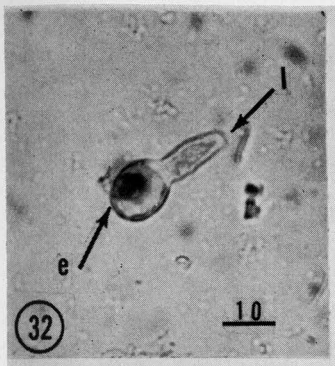
Fig. 35–37. Isolate Var 5; $BBMPTB_{12}$; 2 days after inoculation.

Fig. 38. Isolate HP 4; $BBMPTB_{12}$ aerated with 2–5 % CO_2 in air; 2 weeks after inoculation.

Fig. 40. Isolate Var 5; $BBMPTB_{12}$; 1 week after inoculation.

Fig. 39, 41, 42. Isolate 8–3; $BBMPTB_{12}$; 1 week after inoculation.

Fig. 43. Isolate 8–3; 3 BBMP:1 SS; 2 weeks after inoculation.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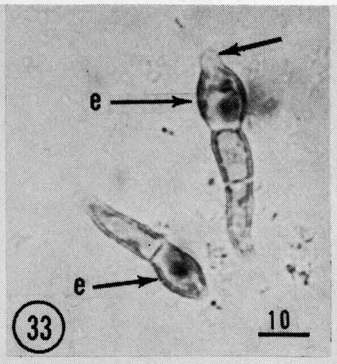
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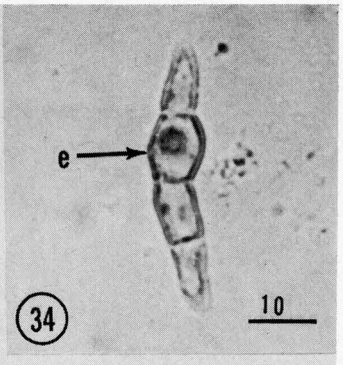
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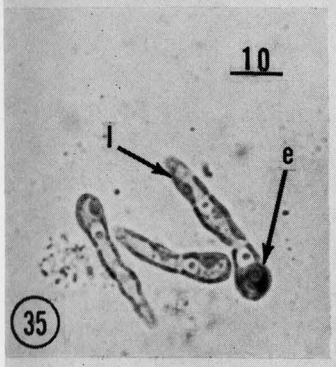
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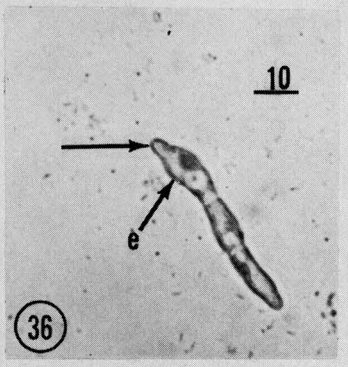
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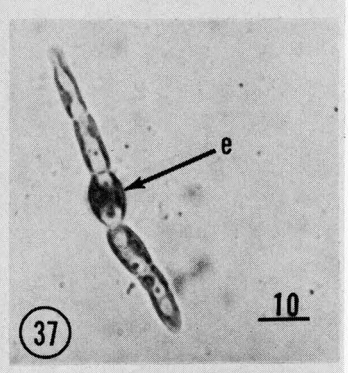
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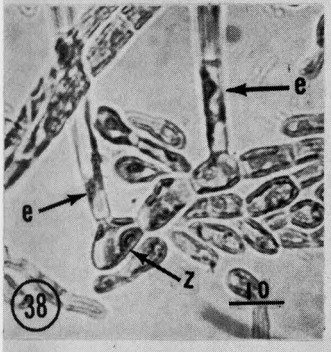
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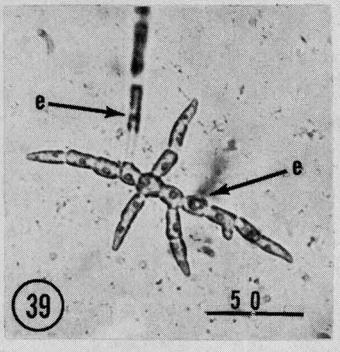
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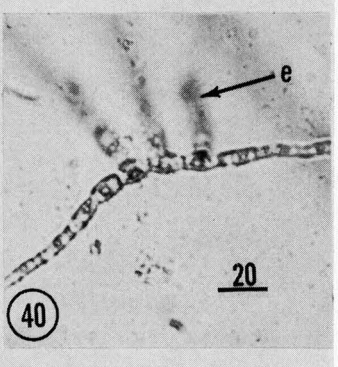
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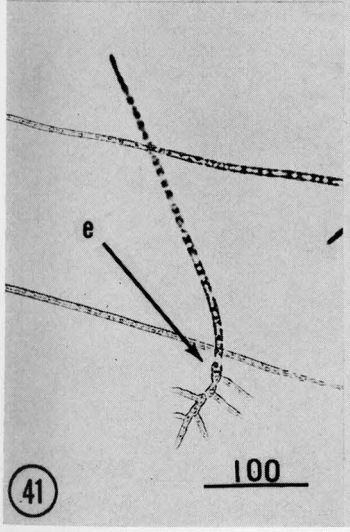
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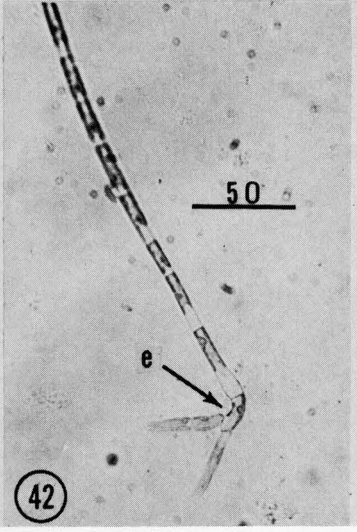
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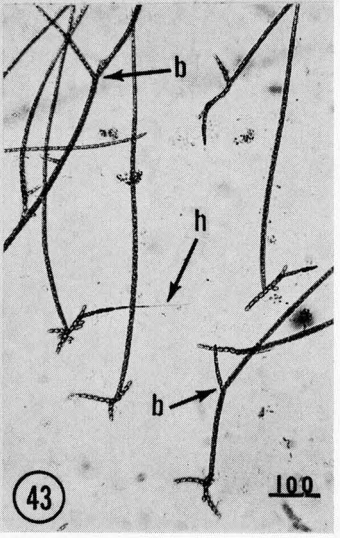
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FIGURES 44–52

***Stigeoclonium aestivale* (Hazen) Collins (Emend.)**
(Size scale in microns)

Fig. 44. Small, filamentous prostrate system with prostrate laterals of restricted growth. Extensive erect system.

Fig. 45, 49. Small, filamentous basal system showing development of rhizoids (r) from ends of prostrate filaments. Extensive erect system.

Fig. 50. Small, filamentous basal system, with rebranching lateral prostrate filaments of restricted growth; site of development of erect filaments (e).

Fig. 51. Small branching basal system composed of akinete-like cells. Extensive alternately branched (b) erect filaments.

Fig. 52. Development of rhizoid (r) from end of fragment of erect filament from the inoculum.

Conditions of culture

Fig. 44. Isolate 8–3; $BBMPTB_{12}$; 1 month after inoculation.

Fig. 45. Isolate 8–3; $BBMPTB_{12}$ aerated with 2–5 % CO_2 in air; 1 month after inoculation.

Fig. 46. Isolate HP 4; $BBMPTB_{12}$ aerated with 2–5 % CO_2 in air; 2 weeks after inoculation.

Fig. 47. Isolate Var 5; $BBMPTB_{12}$; 2 weeks after inoculation.

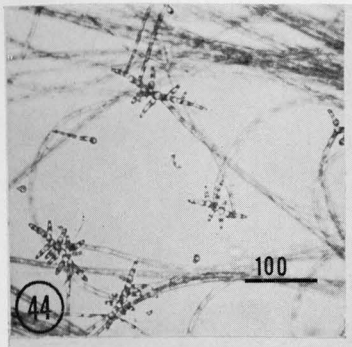
Fig. 48. Isolate 8–3; $BBMPTB_{12}$ aerated with 2–5 % CO_2 in air; 2 weeks after inoculation.

Fig. 49. Isolate HP 4; $BBMPTB_{12}$ aerated with 2–5 % CO_2 in air; 1 month after inoculation.

Fig. 50. Isolate 8–3; $BBMPTB_{12}$; 2 weeks after inoculation.

Fig. 51. Isolate 8–3; 3 BBMP:1 SS; 1 month after inoculation.

Fig. 52. Isolate Var 5; $BBMPTB_{12}$; 5 days after inoculation.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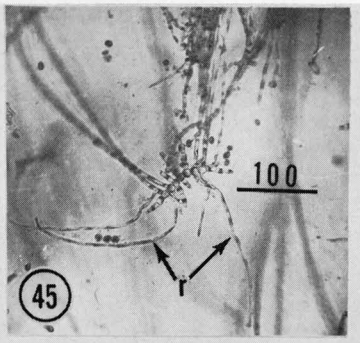
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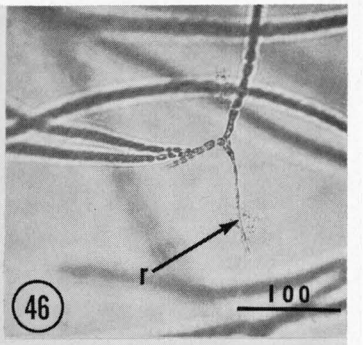
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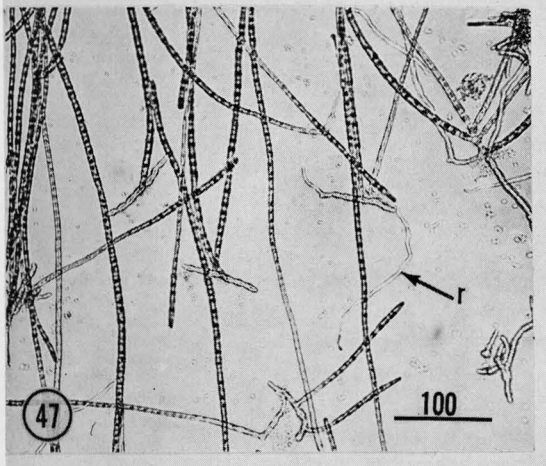
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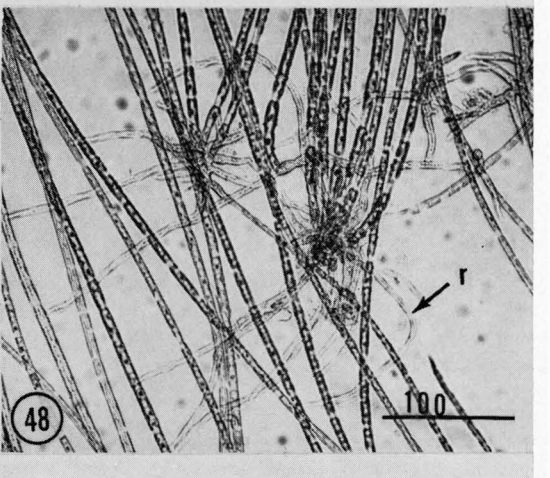
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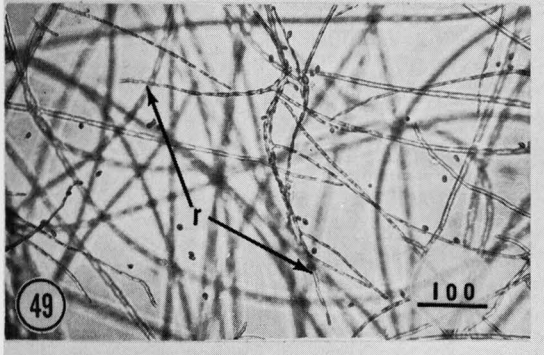
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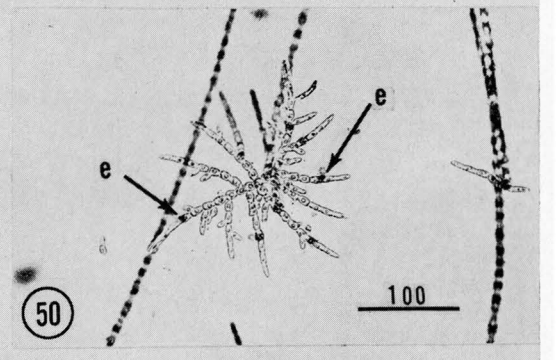
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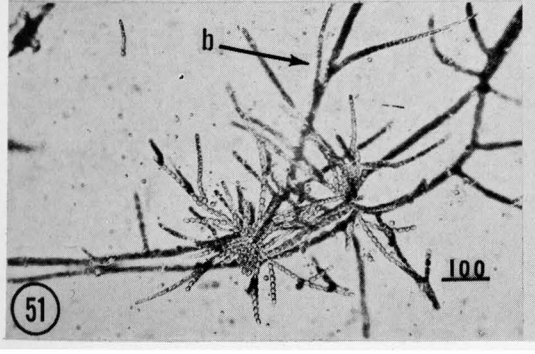
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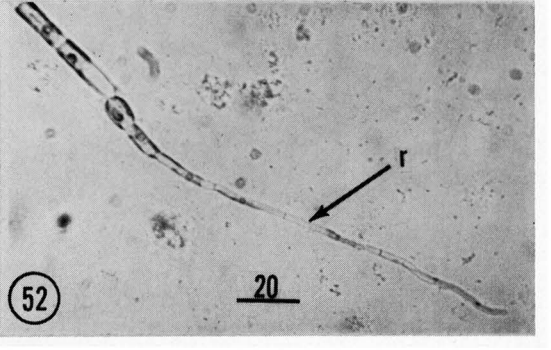
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FIGURES 53–58

***Stigeoclonium aestivale* (Hazen) Collins (Emend.)**
(Size scale in microns)

Fig. 53. Small, filamentous basal system with lateral, prostrate filaments of restricted growth and extensive erect filaments.

Fig. 54. Shortly after release of numerous zoospores (z) from erect filaments.

Fig. 55, 56. Filamentous basal system as grown in Blanco River, San Marcos, Texas. Site of development of erect filaments (e) from prostrate filaments.

Fig. 57. Filamentous basal system; alternate branching (b); multicellular colorless hair (h). Sample taken near surface of culture medium, where medium is evaporating.

Fig. 58. Small, filamentous basal system; extensive erect system; zoospores (z).

Conditions of culture

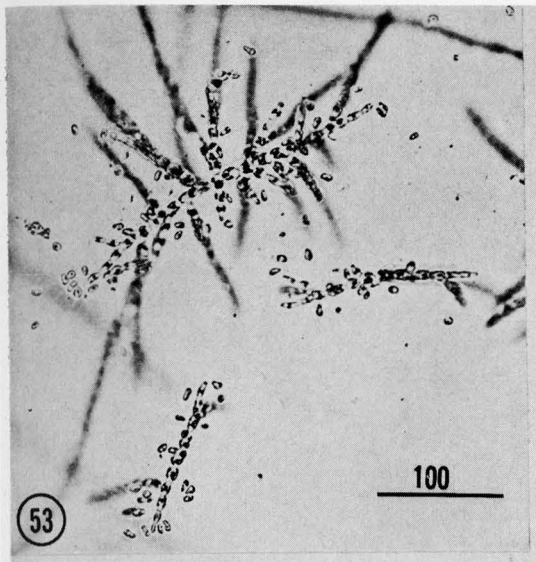
Fig. 53. Isolate Var 5; $BBMPTB_{12}$; 1 week after inoculation.

Fig. 54. Isolate Var 5; $BBMPTB_{12}$; 2 days after transfer to fresh medium.

Fig. 55. Isolate 8–3; 1 week $BBMPTB_{12}$ in laboratory, 2 weeks Blanco River, San Marcos, Texas.

Fig. 56. Isolate Var 5; 1 week $BBMPTB_{12}$ in laboratory, 2 weeks Blanco River, San Marcos, Texas.

Fig. 57–58. Isolate Var 5; $BBMPTB_{12}$; 1 month after inoculation.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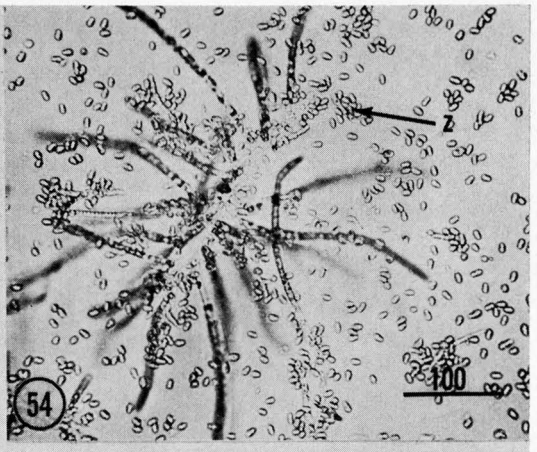
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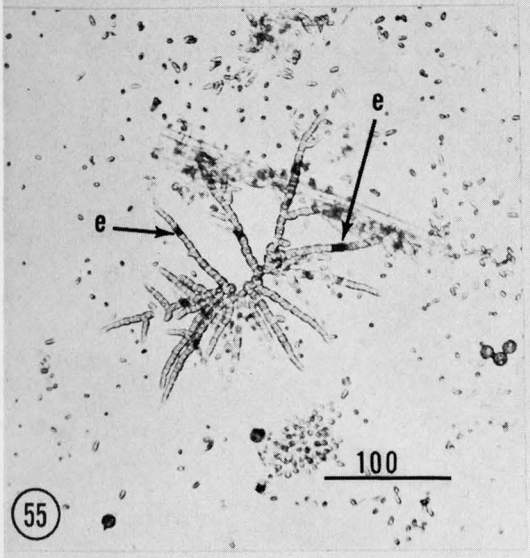
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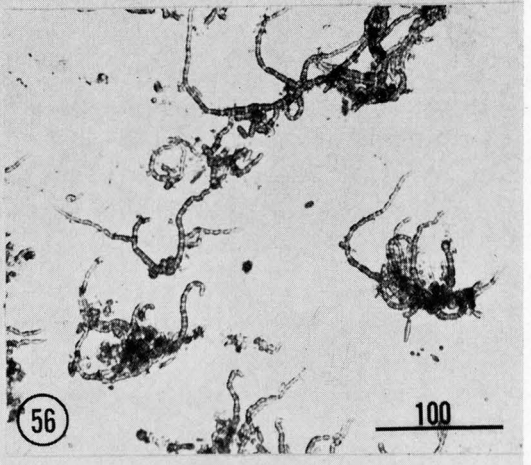
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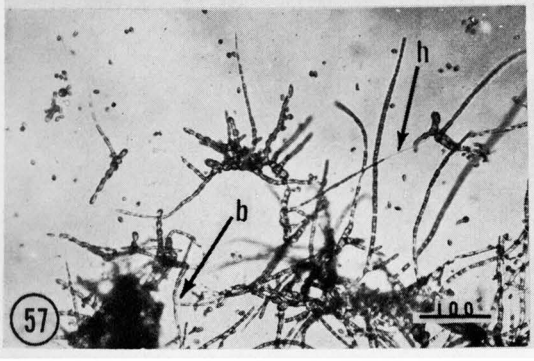
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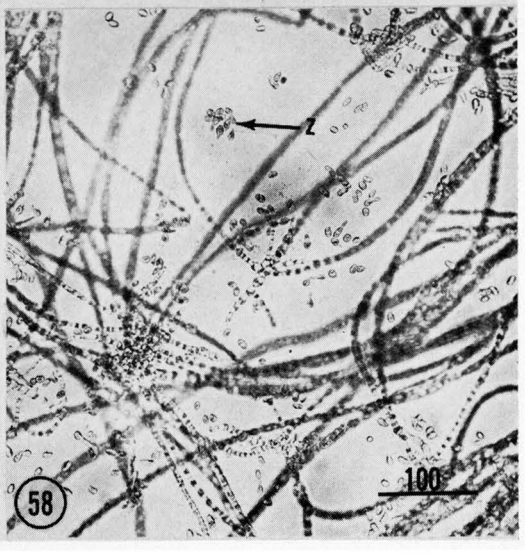
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FIGURES 59–66

***Stigeoclonium aestivale* (Hazen) Collins (Emend.)**
(Size scale in microns)

Fig. 59, 60. Cylindrical, non-constricted cells of actively growing erect filaments.

Fig. 61. Intercalary division (arrow) of cells of erect filaments; slight constriction at partition walls.

Fig. 62–64. Formation of branch following diagonal division of cell contents (arrows).

Fig. 65. Unbranched erect filaments in young culture.

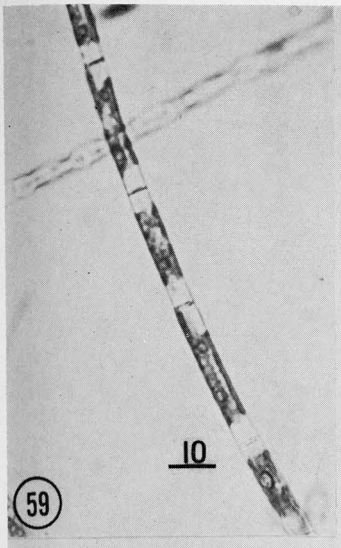
Fig. 66. Random, secund branching from older erect filaments.

Conditions of culture

Fig. 59–61. Isolate HP 4; $BBMPTB_{12}$ aerated with 2–5 % CO_2 in air; 2 weeks after inoculation.

Fig. 62–65. Isolate Var 5; $BBMPTB_{12}$ aerated with 2–5 % CO_2 in air; 2 weeks after inoculation.

Fig. 66. Isolate Var 5; $BBMPTB_{12}$; 1 month after inoculation.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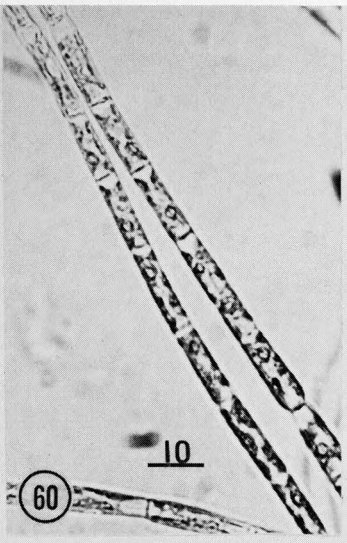
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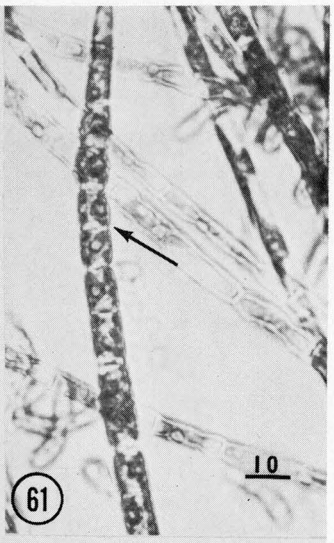
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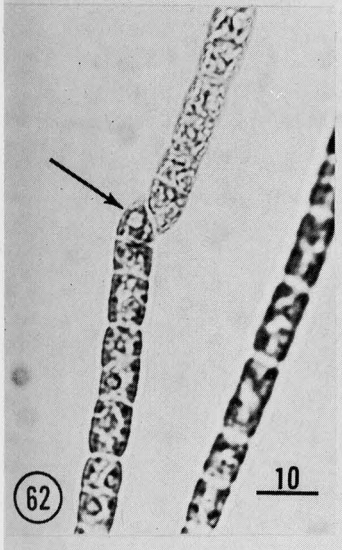
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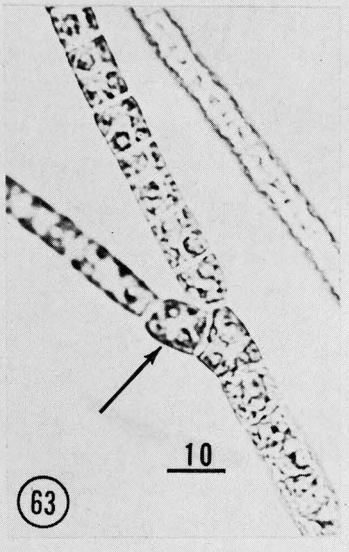
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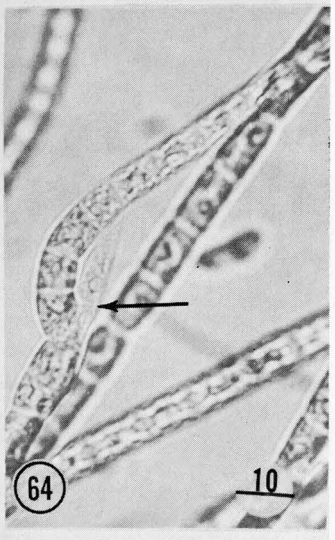
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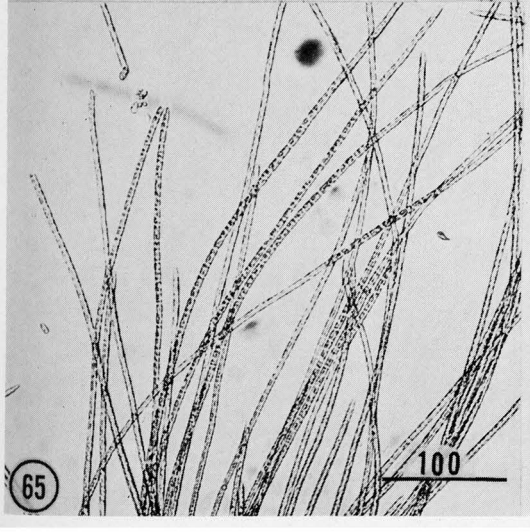
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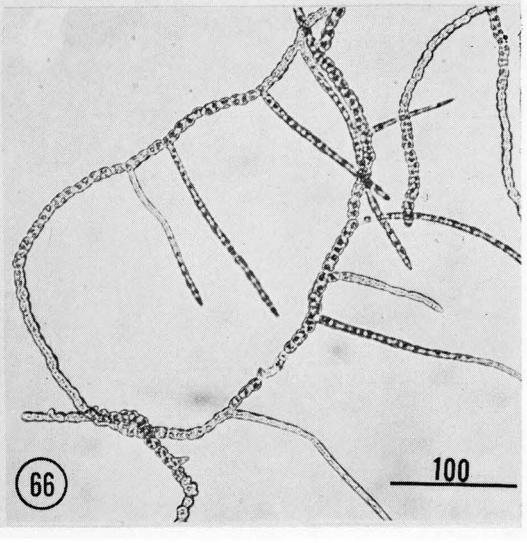

100
66

FIGURES 67–72

***Stigeoclonium aestivale* (Hazen) Collins (Emend.)**
(Size scale in microns)

Fig. 67. Random, secund branching from older erect filaments; empty cells (ec) after release of zoospores (z).

Fig. 68. Sharply pointed and blunt branch tips (arrows).

Fig. 69–72. Thick-walled (w), starch-filled, akinete-like cells.

Fig. 69. Cells dissociating in groups of 2 (arrow).

Fig. 70. Very branched filaments.

Conditions of culture

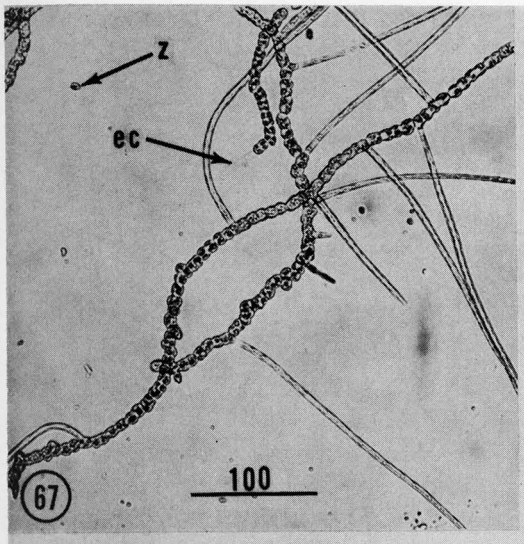
Fig. 67. Isolate Var 5; BBMPTB$_{12}$; 1 month after inoculation.

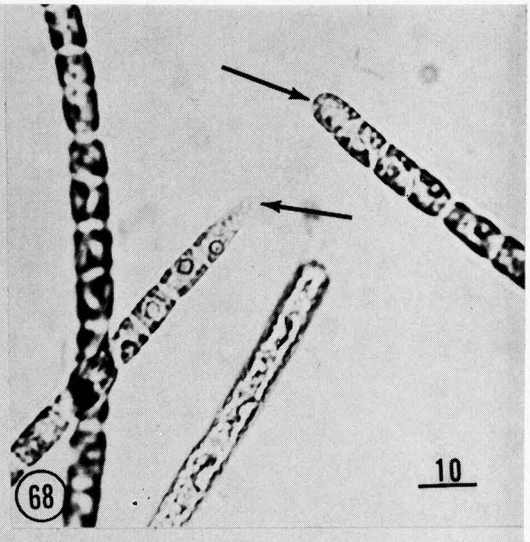
Fig. 68. Isolate Var 5; BBMPTB$_{12}$ aerated with 2–5 % CO_2 in air; 2 weeks after inoculation.

Fig. 69. Isolate 8–3; 1.5 % BBMPTB$_{12}$ agar; 2 months after inoculation.

Fig. 70–71. Isolate HP 4; 1.5 % BBMPTB$_{12}$ agar; 2 months after inoculation.

Fig. 72. Isolate Var 5; 1.5 % BBMPTB$_{12}$ agar; 2 months after inoculation.


z
ec
100
67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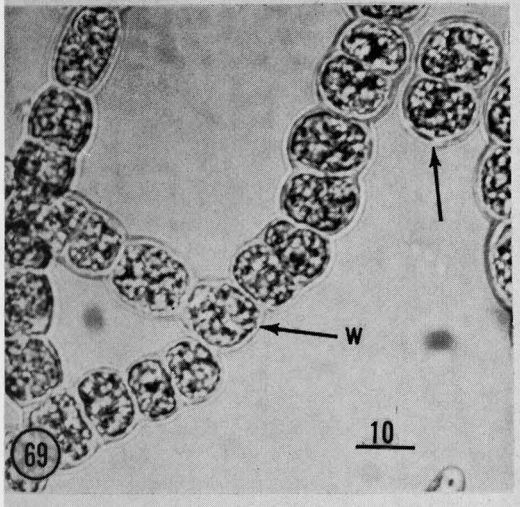
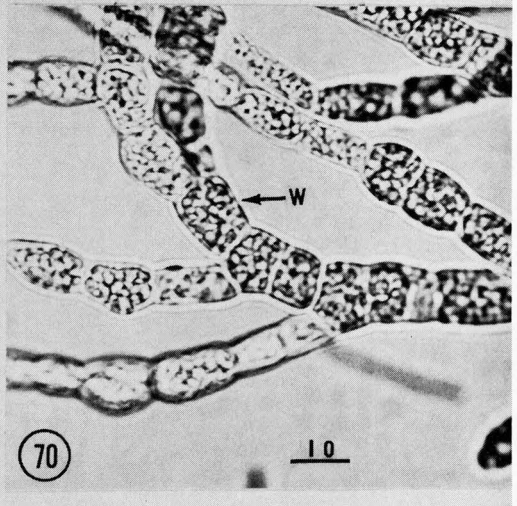
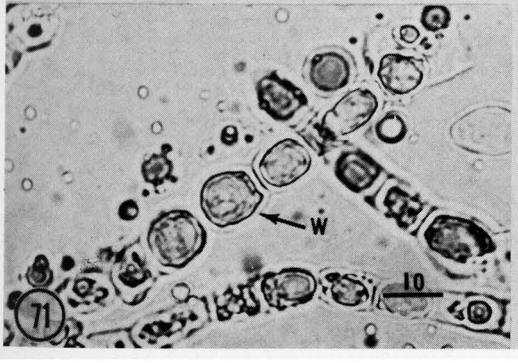
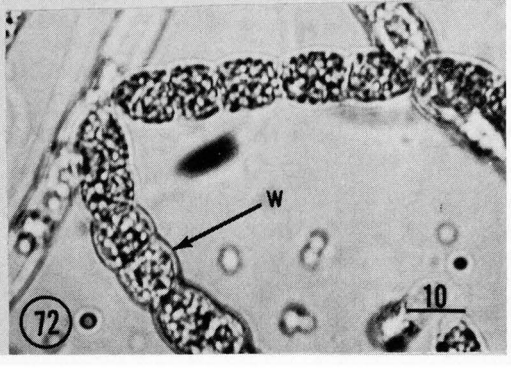

W
10
69


W
10
70


W
10
71


W
10
72

FIGURES 73–77

***Stigeoclonium aestivale* (Hazen) Collins (Emend.)**

Fig. 73, 74. Thick-walled (w), starch-filled, akinete-like cells with bulbous ends (arrow) and multicellular colorless hairs (h) (Size scale in microns.)

Fig. 75. Dissociation of filaments into unicellular akinete-like cells (Size scale in microns.)

Fig. 76. Center of colony on agar (× 24).

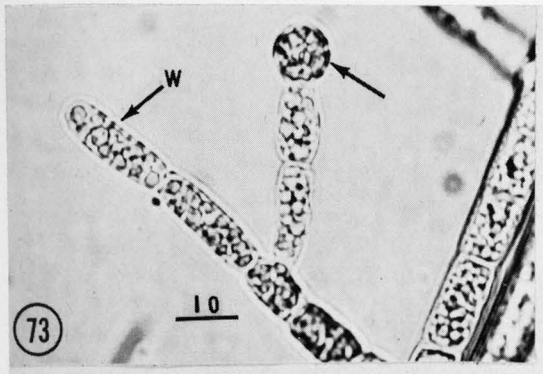
Fig. 77. Edge of flat, vermiform colony on agar (× 24).

Conditions of culture

Fig. 73–75. Isolate HP 4; 1.5 % BBMPTB$_{12}$ agar; 2 months after inoculation.

Fig. 76. Isolate Var 5; 1.5 % BBMPTB$_{12}$ agar; 1 month after inoculation.

Fig. 77. Isolate 8–3; 1.5 % BBMPTB$_{12}$ agar; 1 month after inoculation.


W
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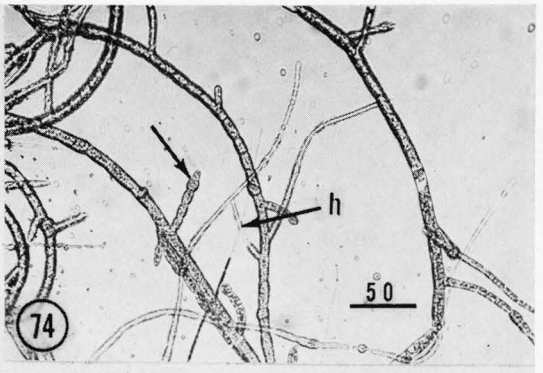
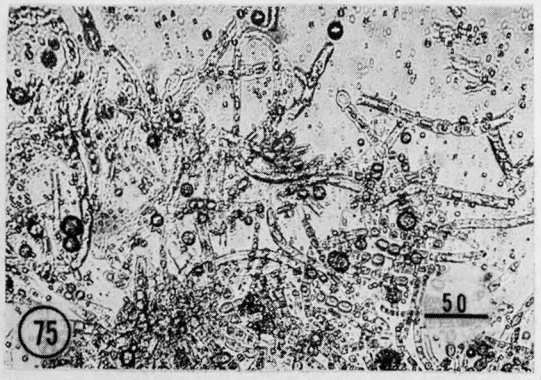

h
50
74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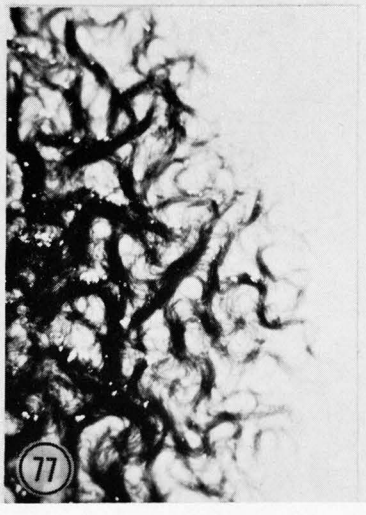

77

FIGURES 78–83

***Stigeoclonium subsecundum* (Kütz.) Kützing**
(Size scale in microns)

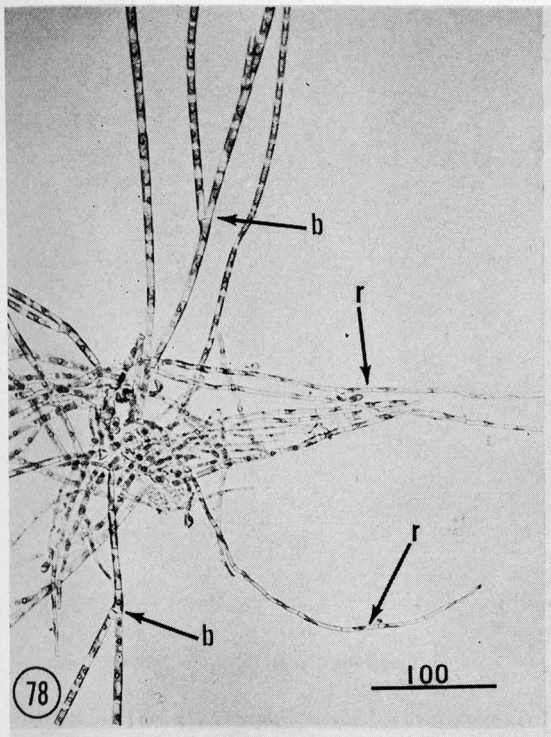
Fig. 78–83. Small, filamentous basal system with short, prostrate lateral branches (l) and numerous spreading rhizoids (r) developing from the cells of the prostrate system; extensive erect filaments (e); alternate branches (b) from cylindrical, non-constricted cells of the erect filaments.

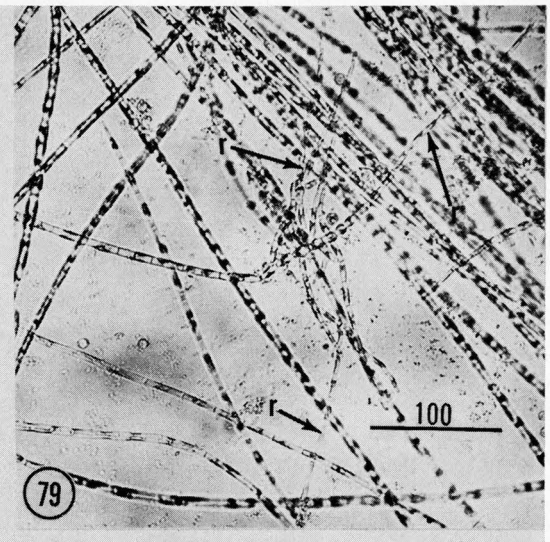
Conditions of culture

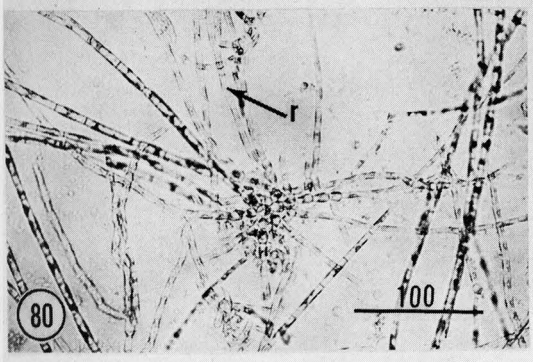
Fig. 78, 81. Isolate 19–11–V; $BBMPTB_{12}$; 2 weeks after inoculation.

Fig. 79, 80. Isolate 19–11–V; $BBMPTB_{12}$; 1 month after inoculation.

Fig. 82, 83. Isolate 19–11–V; 1 week $BBMPTB_{12}$ in laboratory, 2 weeks Blanco River at San Marcos, Texas.


b
r
r
b
100
78


r
r
100
79


r
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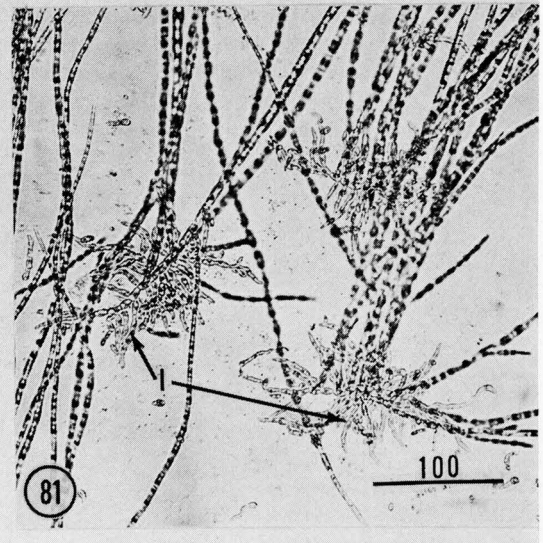
80


l
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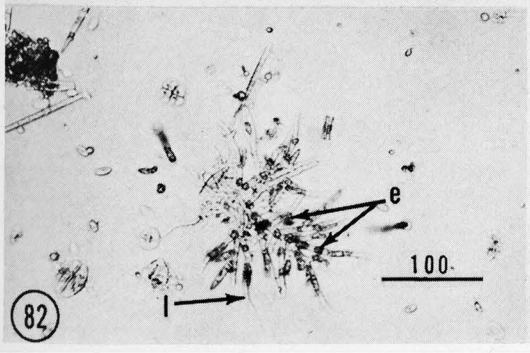
81


e
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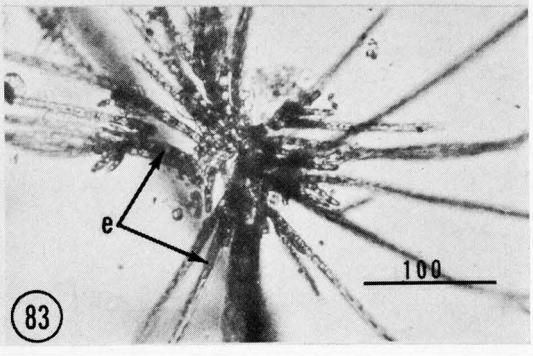
l
82


e
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83

FIGURES 84–91

***Stigeoclonium subsecundum* (Kütz.) Kützing**
(Size scale in microns)

Fig. 84, 85. Alternate branches which develop from top of cell near the septum by enation of cell contents (fb); long spreading rhizoids (r) from small barrel-shaped cells (c); branch tips blunt (bl) or with multicellular colorless hairs (h).

Fig. 86–88. Alternate or secund branching from actively growing erect filaments; branch tips with acute or sharp points; downward-growing rhizoid (r) from cylindrical cells (Fig. 86).

Fig. 89, 90. Alternate branching of erect filaments; branch tips with sharp points (s) or multicellular colorless hairs (h).

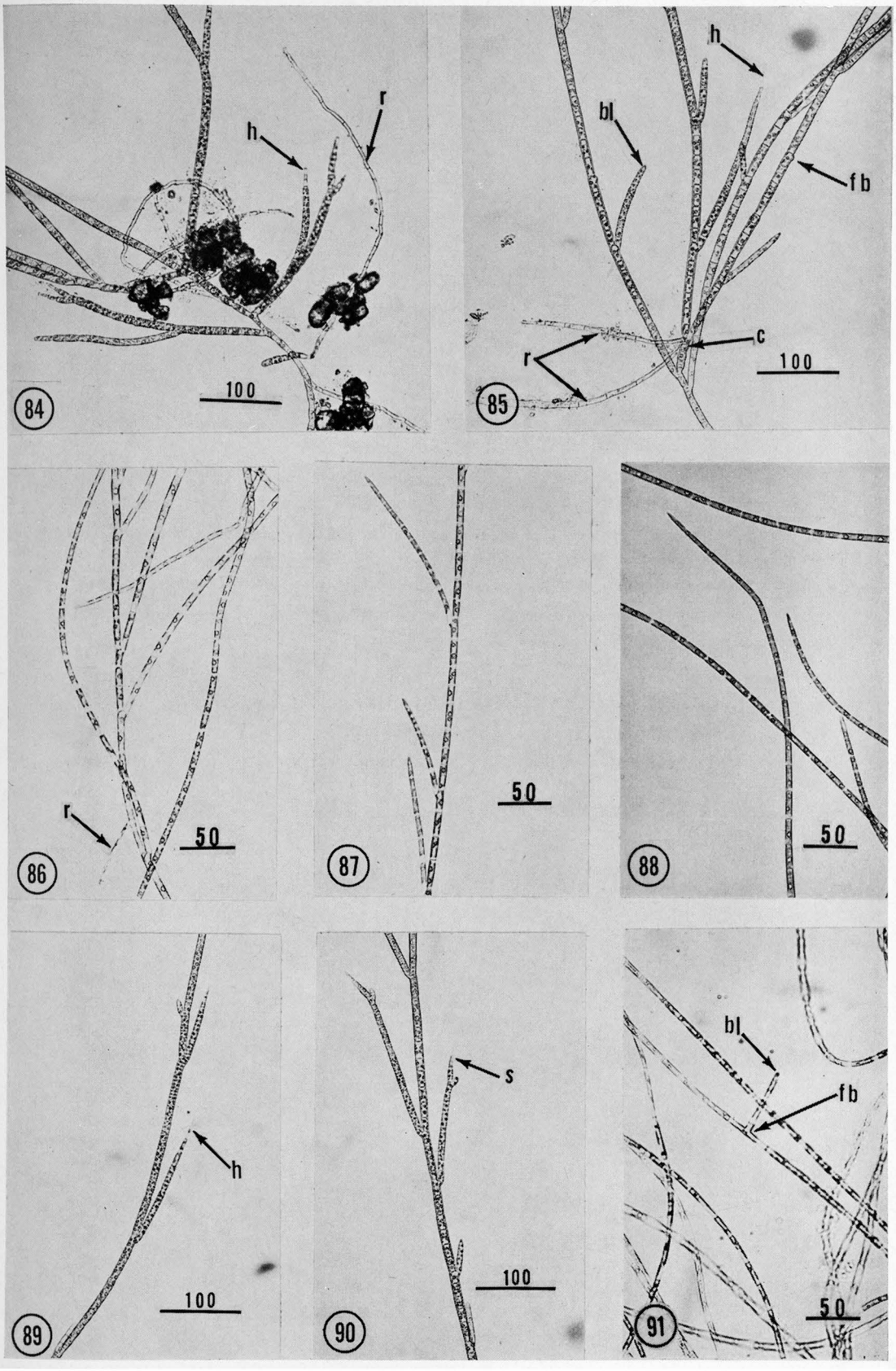
Fig. 91. Branch formation from middle of cell (fb); branch tip blunt (bl).

Conditions of culture

Fig. 84, 85, 89, 90. Isolate 19–11–V; 1 week $BBMPTB_{12}$ in laboratory, 2 weeks Blanco River, San Marcos, Texas.

Fig. 86–88. Isolate 19–11–V; $BBMPTB_{12}$ aerated with 2–5 % CO_2 in air; 3 weeks after inoculation.

Fig. 91. Isolate 19–11–V; $BBMPTB_{12}$; 1 month after inoculation.


h
r
100
84
h
bl
fb
c
r
100
85
r
50
86
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87
50
88
h
100
89
s
100
90
bl
fb
50
91

FIGURES 92–96

***Stigeoclonium subsecundum* (Kütz.) Kützing**

Fig. 92, 93. This isolate did not form, upon aging, the starch-filled, thick-walled, akinete-like cells characteristic of other isolates (size scale in microns).

Fig. 94. Development of rhizoids (r) from ends of fragment of erect filaments (size scale in microns).

Fig. 95. Zoospore formation; 2 zoospores in each cell, released in vesicle (size scale in microns).

Fig. 96. Edge of wavy or vermiform colony on agar (× 24).

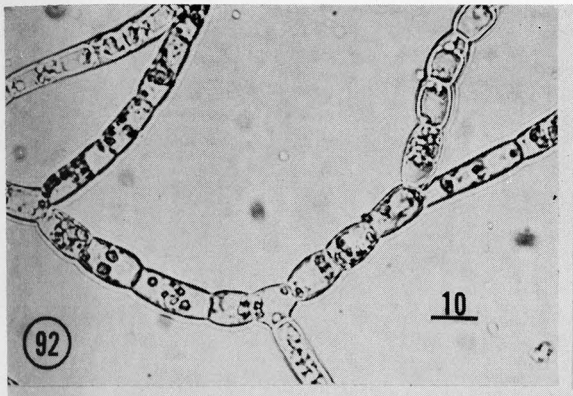
Conditions of culture

Fig. 92, 93. Isolate 19–11–V; 1.5 % $BBMPTB_{12}$ agar; 2 months after inoculation.

Fig. 94. Isolate 19–11–V; $BBMPTB_{12}$; 5 days after inoculation.

Fig. 95. Isolate 19–11–V; 1 week $BBMPTB_{12}$ in the laboratory, 2 weeks in Blanco River, San Marcos, Texas.

Fig. 96. Isolate 19–11–V, 1.5 % $BBMPTB_{12}$ agar; 1 month after inoculation.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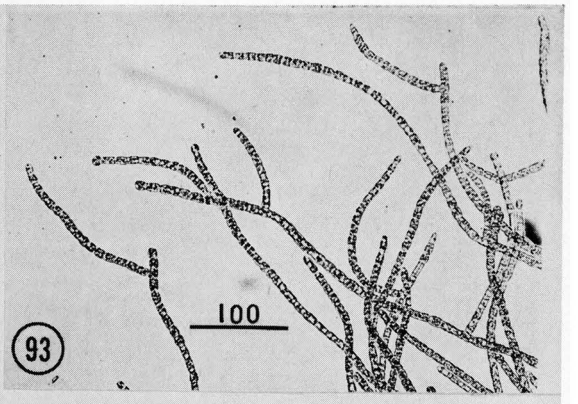
92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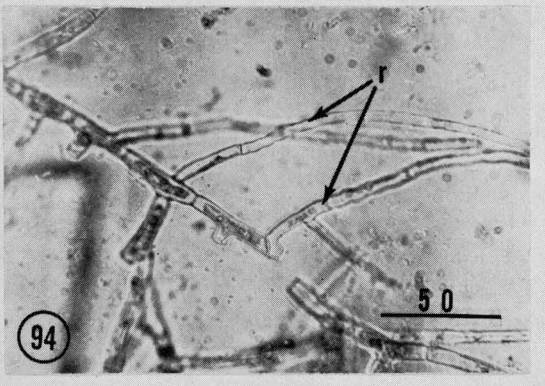
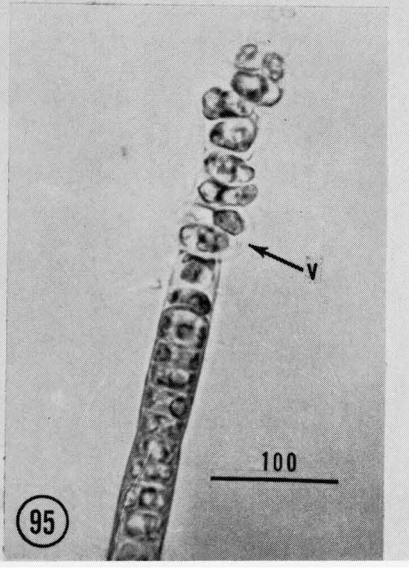

r
50
94


v
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96

FIGURES 97–104

***Stigeoclonium tenue* (Ag.) Kütz. (Emend.)**
(Size scale in microns)

Fig. 97–99. Zoospore formation. Some cells slightly swollen (s); cell contents divided horizontally or occasionally obliquely (arrows); empty cell from which zoospore has escaped (ec); escaping zoospore (z).

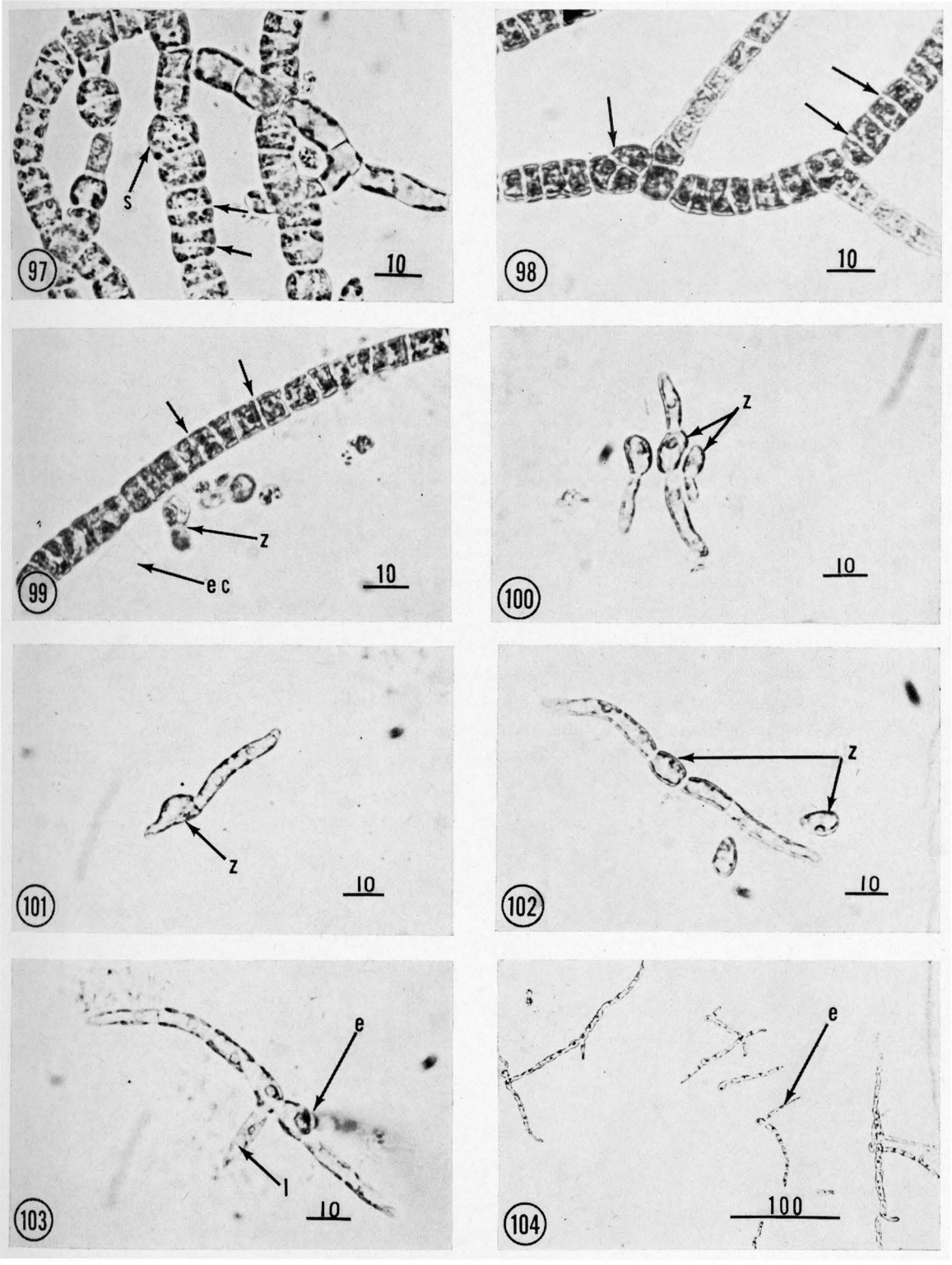
Fig. 100–104. Zoospore germination and early germling formation. Prostrate filaments developed unilaterally and subsequently bilaterally from the zoospore (z) to form a basal filament from which lateral prostrate filaments (l) developed. Erect filaments (e) developed from the original zoospore.

Conditions of culture

Fig. 97–99. Isolate 19–1–E; $BBMPTB_{12}$ aerated with 2–5 % CO_2 in air; 2 weeks after inoculation.

Fig. 100–103. Isolate 6–1D; $BBMPTB_{12}$ aerated with 2–5 % CO_2 in air; 2 weeks after inoculation.

Fig. 104. Isolate Var I; $BBMPTB_{12}$; 1 week after inoculation.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104
e
100

FIGURES 105–110

***Stigeoclonium tenue* (Ag.) Kütz. (Emend.)**
(Size scale in microns)

Fig. 105, 106. Early stage in development of prostrate thallus; lateral prostrate filaments (l) and erect filaments (e) from cells of first basal filament.

Fig. 107, 108, 110. Lateral prostrate filaments nearly as long as first basal filament; cells of basal system globular and constricted (g); rhizoidal tips (r) of lateral filaments.

Fig. 109. Globular and constricted cells of basal system (g).

Conditions of culture

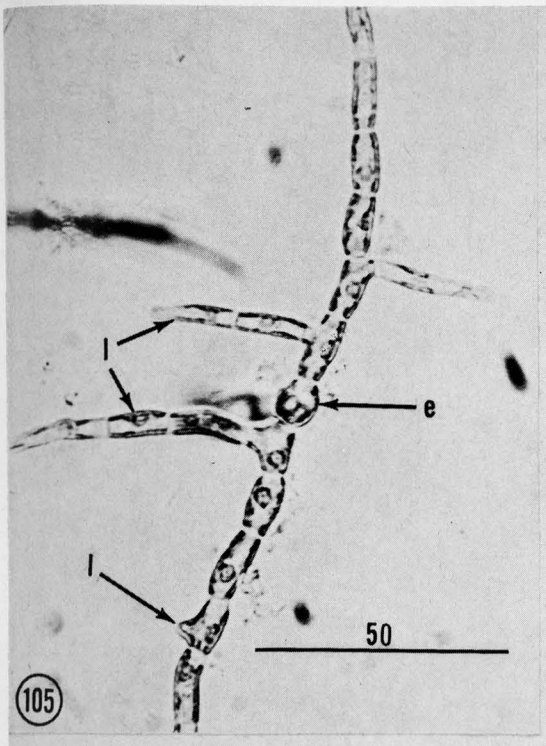
Fig. 105. Isolate 6–1D; BBMPTB$_{12}$ aerated with 2–5 % CO_2 in air; 2 weeks after inoculation.

Fig. 106. Isolate 6–1D; BBMPTB$_{12}$; 1 week after inoculation.

Fig. 107, 108. Isolate 19–1–E; BBMPTB$_{12}$; 1 week after inoculation.

Fig. 109. Isolate 6–1D; BBMPTB$_{12}$; 1 month after inoculation.

Fig. 110. Isolate 6–1D; BBMPTB$_{12}$ aerated with 2–5 % CO_2 in air; 1 month after inoculation.



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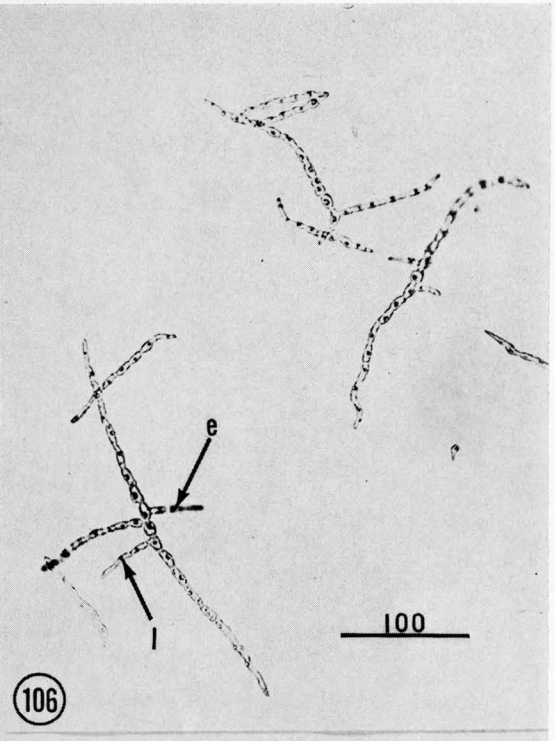

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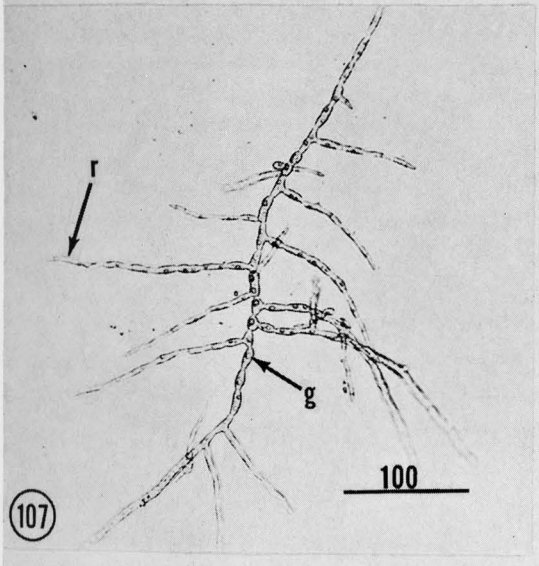

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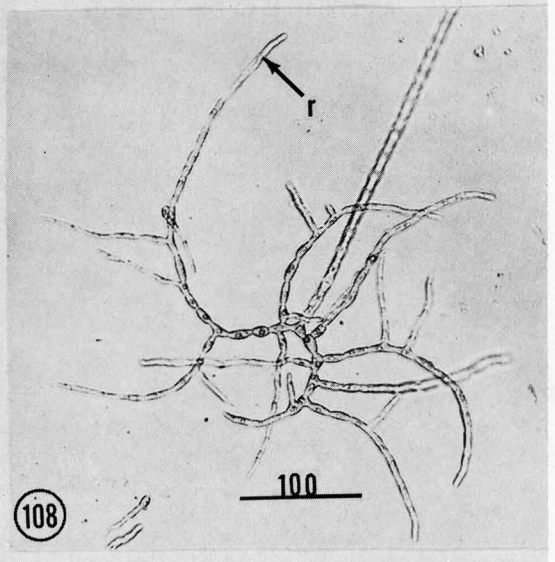

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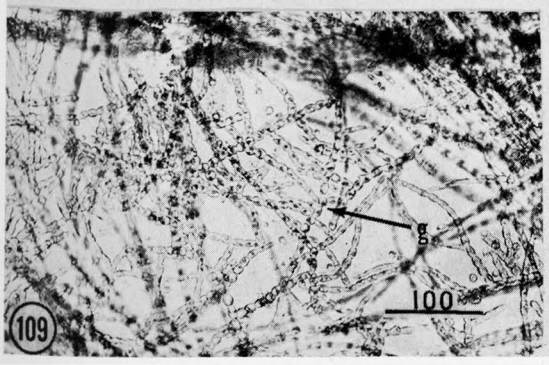

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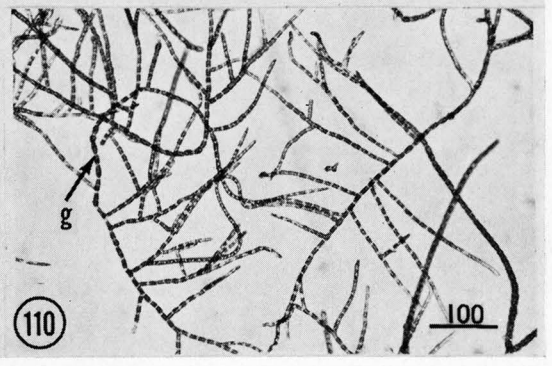


110

FIGURES 111–118

***Stigeoclonium tenue* (Ag.) Kütz. (Emend.)**
(Size scale in microns)

Fig. 111. Basal system with rebranching prostrate lateral filaments (l); alternately branched erect filaments (b) with sharp-pointed branch tips (t).

Fig. 112. Several prostrate thalli.

Fig. 113, 114. Adjacent development of several zoospores to form an "aggregate" thallus. It is impossible to determine clearly the morphology of a single thallus. Cells of basal system globular and constricted (g); rhizoidal tips of lateral prostrate filaments (r).

Fig. 115. Single thallus (as above). Globular and constricted cells of basal system (g); long lateral prostrate branches with rhizoidal tips (r); erect filaments (e).

Fig. 116. Development of rhizoids (r) from fragments of erect filaments (probably from inoculum).

Fig. 117–118. Adjacent development of several plants. Basal system more compact; cells very round and akinete-like.

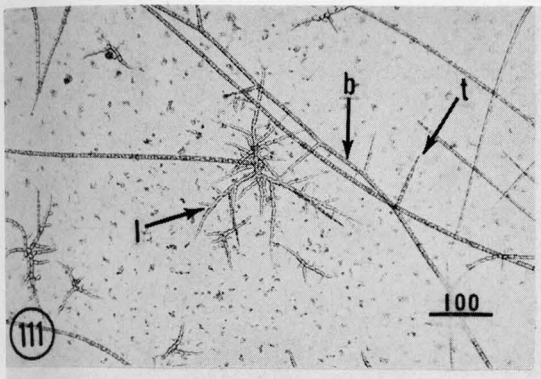
Conditions of culture

Fig. 111. Isolate Var I; 3 BBMP:1 SS; 1 week after inoculation.

Fig. 112–116. Isolate Var I; $BBMPTB_{12}$; 2 weeks after inoculation.

Fig. 117. Isolate 6–1D; 3 BBMP:1 SS; 2 weeks after inoculation.

Fig. 118. Isolate 19–1–E; 2 BBMP:1 SS; 2 weeks after inoculation.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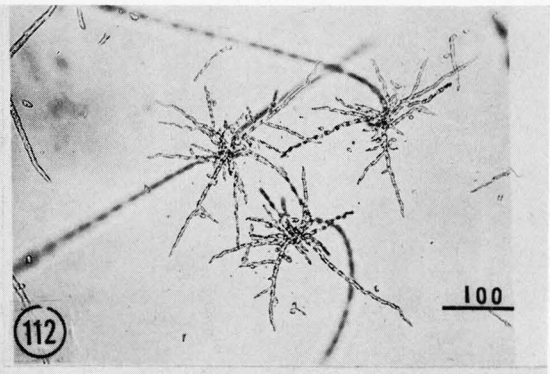
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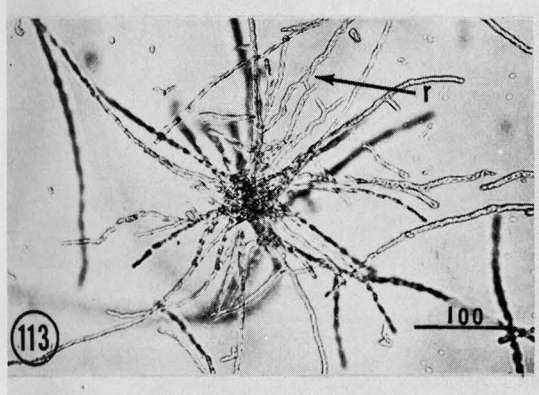
r
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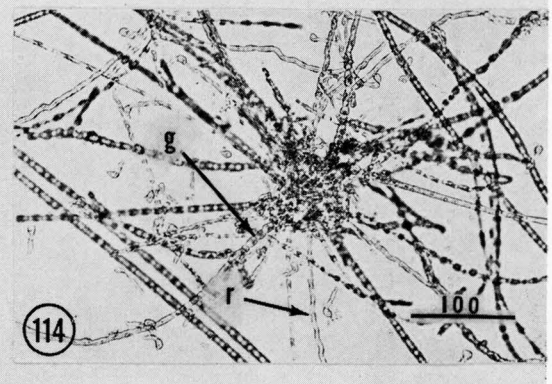
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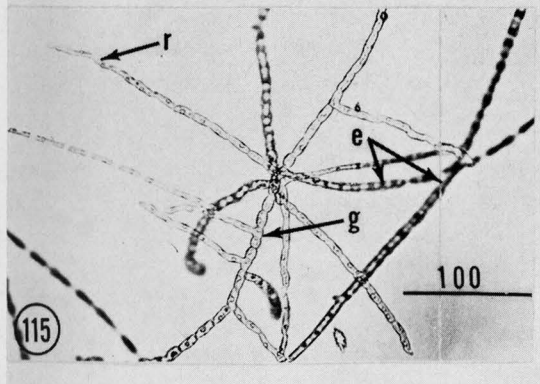
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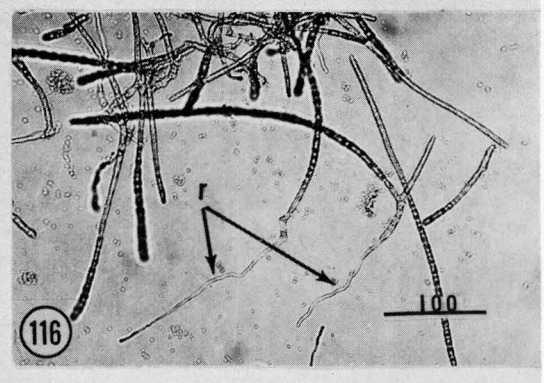
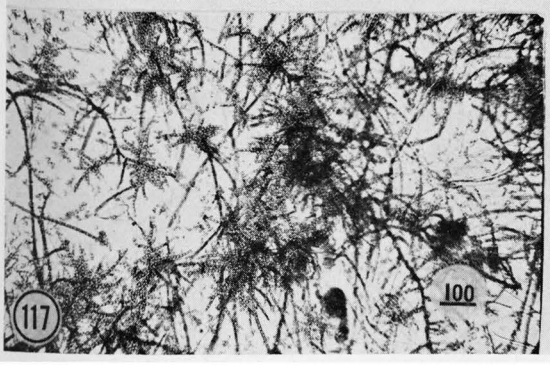
115


r
100
116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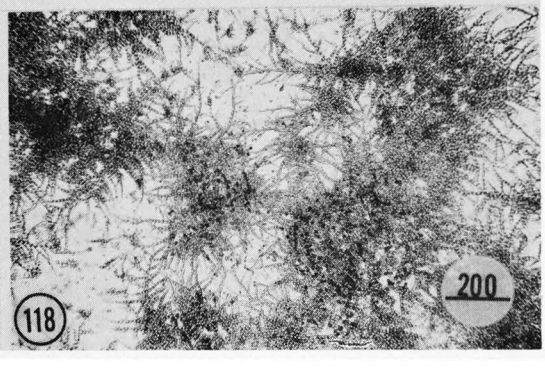

200
118

FIGURES 119–124

***Stigeoclonium tenue* (Ag.) Kütz. (Emend.)**
(Size scale in microns)

Fig. 119, 120. Young plants at interface between culture medium and air, before extensive development of lateral prostrate branches.

Fig. 119. Single thallus.

Fig. 120. Several thalli.

Fig. 121–123. Typical spreading prostrate thalli. Globular and constricted cells of prostrate thallus (g); long lateral branches with rhizoidal tips (r).

Fig. 124. "Corkscrew" or curved rhizoidal tip (r) of lateral prostrate branch.

Conditions of culture

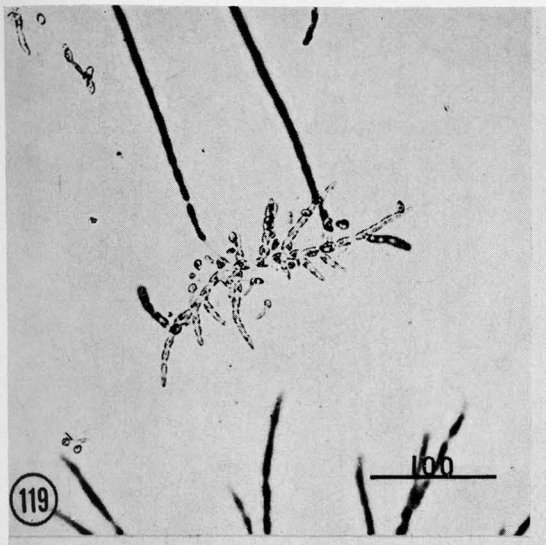
Fig. 119, 120. Isolate Gold; $BBMPTB_{12}$; 1 week after inoculation.

Fig. 121. Isolate 19–1–E; $BBMPTB_{12}$; 1 week after inoculation.

Fig. 122. Isolate 19–1–E; $BBMPTB_{12}$; 3 weeks after inoculation.

Fig. 123. Isolate Gold; $BBMPTB_{12}$; 3 weeks after inoculation.

Fig. 124. Isolate 6–1D; $BBMPTB_{12}$ aerated with 2–5 % CO_2 in air; 2 weeks after inoculation.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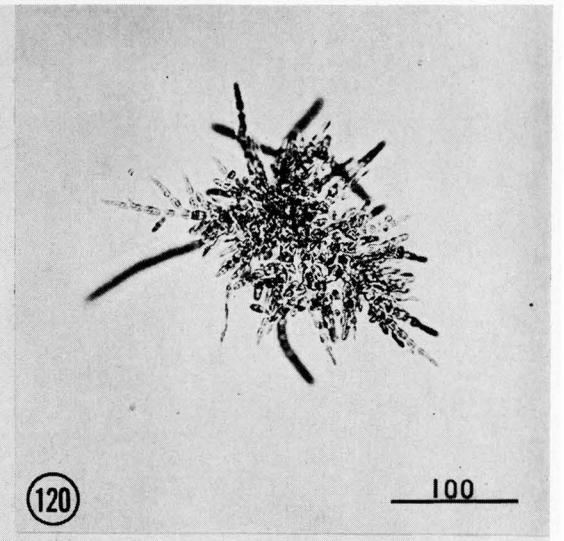
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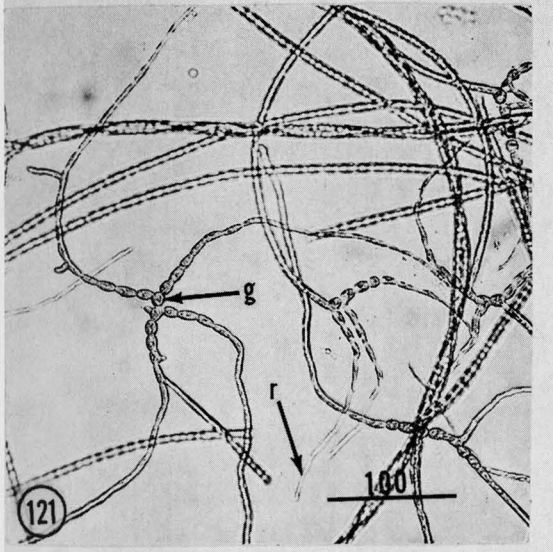
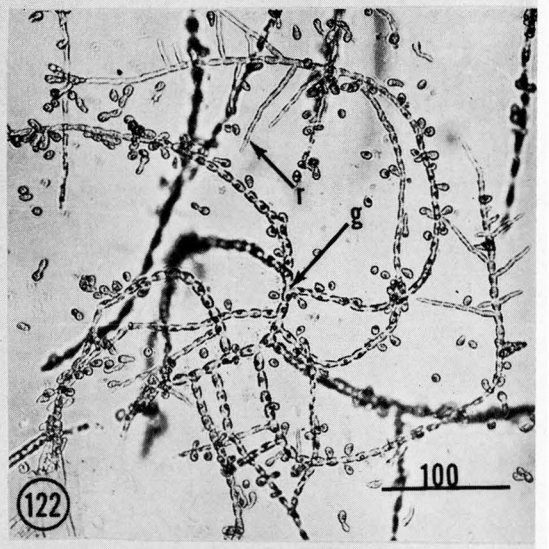

g
r
100
121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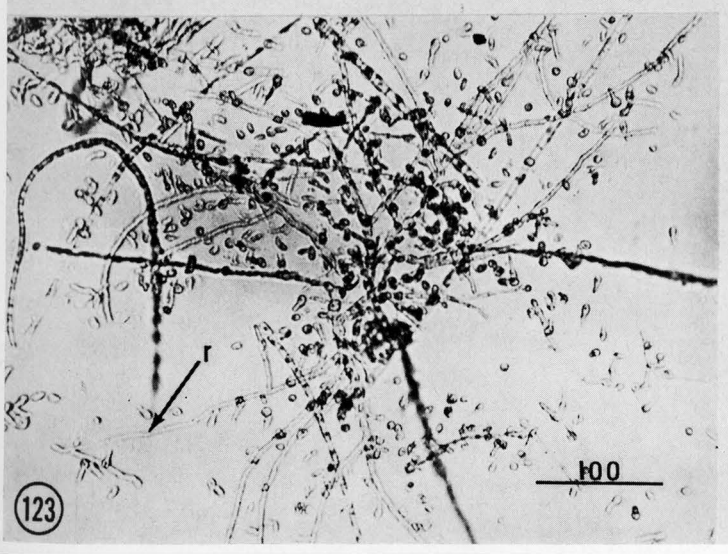
122


r
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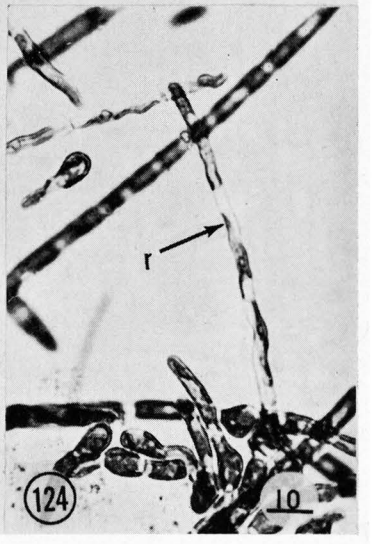
123


r
10
124

FIGURES 125–130

***Stigeoclonium tenue* (Ag.) Kütz. (Emend.)**
(Size scale in microns)

Fig. 125. Several thalli. Typical spreading filamentous basal system; long, lateral prostrate branches with rhizoidal tips (r).

Fig. 126–128. Formation of akinetes by cells of the basal system.

Fig. 129. Rhizoidal tip (r) of prostrate lateral filament.

Fig. 130. Alternate branching (arrow) and secund branching (arrow).

Conditions of culture

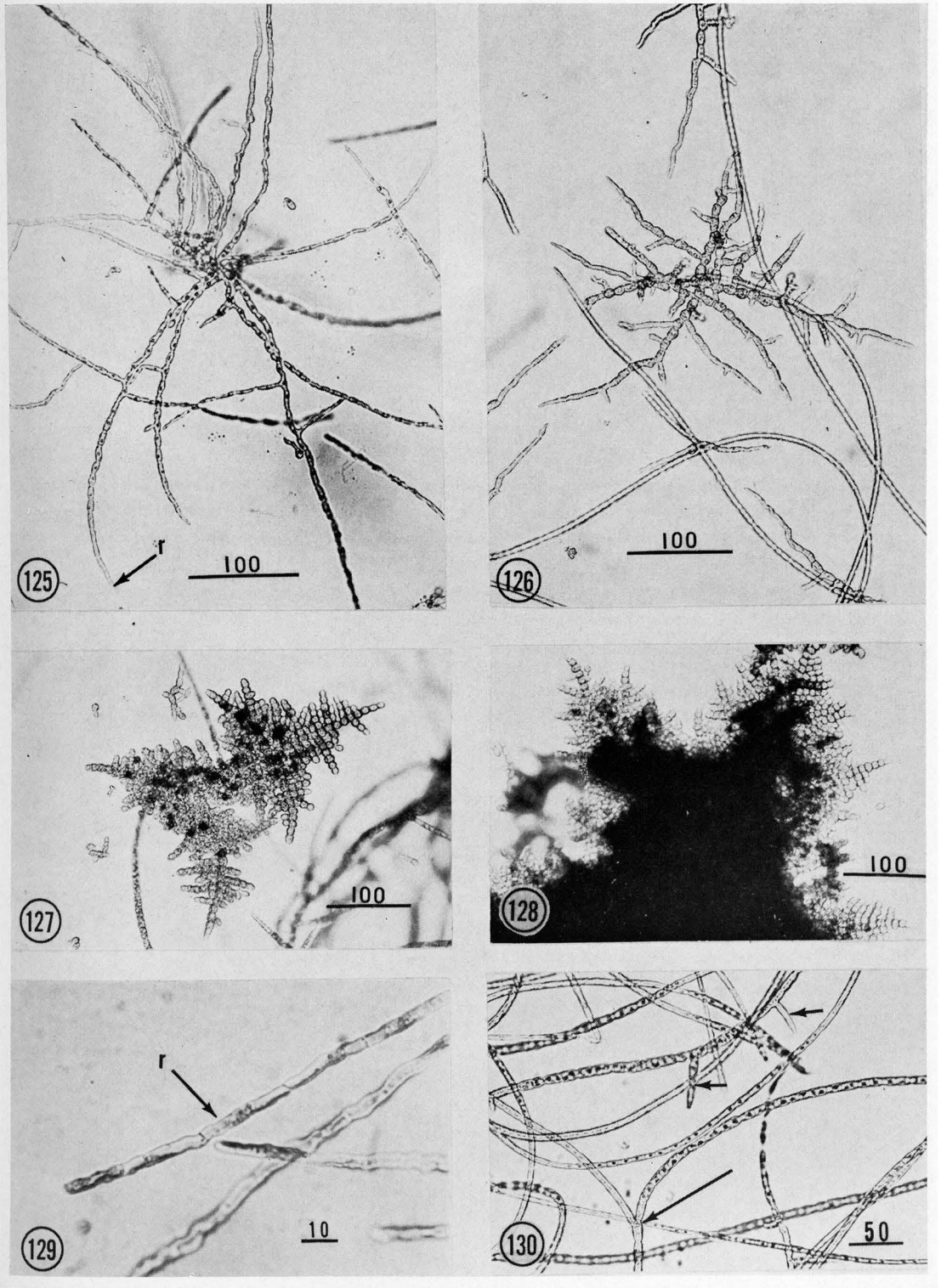
Fig. 125, 126. Isolate 6–1D; BBMPTB$_{12}$; 2 weeks after inoculation.

Fig. 127. Isolate 6–1D; 3 BBMP: 1 SS; 2 weeks after inoculation.

Fig. 128. Isolate 6–1D; 1 week BBMPTB$_{12}$ in the laboratory, 2 weeks Blanco River, San Marcos, Texas.

Fig. 129. Isolate Var I; BBMPTB$_{12}$ aerated with 2–5 % CO_2 in air; 1 month after inoculation.

Fig. 130. Isolate 6–1D; BBMPTB$_{12}$; 1 month after inoculation.


125
r
100
126
100
127
100
128
100
129
r
10
130
50

FIGURES 131–136

***Stigeoclonium tenue* (Ag.) Kütz. (Emend.)**
(Size scale in microns)

Fig. 131, 132. Alternate-dichotomous branching of erect filaments (arrows) which terminated in multicellular colorless hairs (h).

Fig. 133. Rhizoidal tip (r) of erect filament; alternate branching (b). Photograph taken at the evaporation level of the medium.

Fig. 134. Erect filaments with constricted or barrel-shaped cells and alternate branching.

Fig. 135. Branch tip with multicellular colorless hair (h).

Fig. 136. Thick-walled (w) akinete.

Conditions of culture

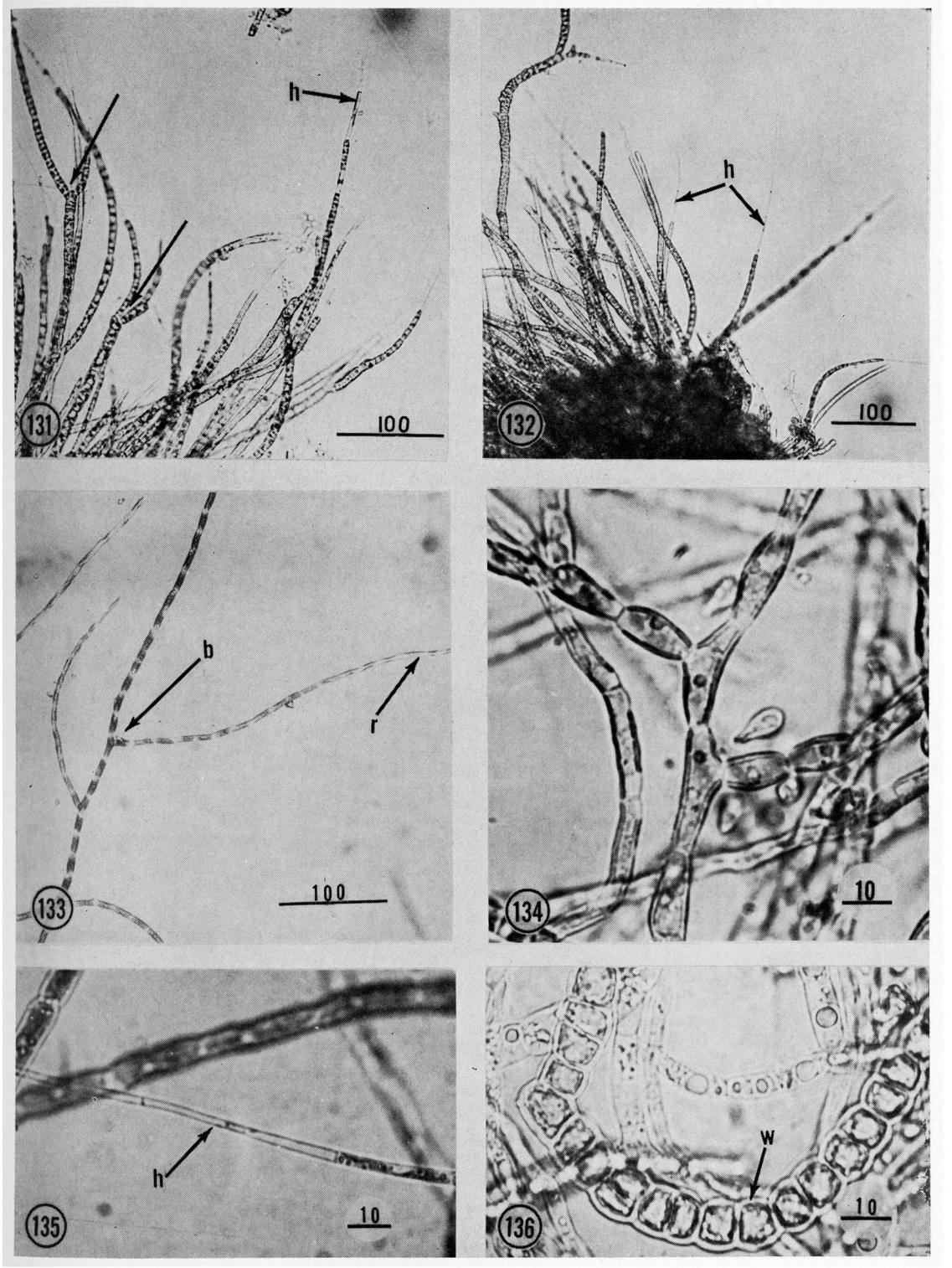
Fig. 131, 132. Isolate 6–1D; 1 week $BBMPTB_{12}$ in laboratory, 2 weeks in Blanco River, San Marcos, Texas.

Fig. 133. Isolate Var I; $BBMPTB_{12}$ aerated with 2–5 % CO_2 in air; 3 weeks after inoculation.

Fig. 134. Isolate 19–1–E; $BBMPTB_{12}$; 2 weeks after inoculation.

Fig. 135. Isolate Var I; $BBMPTB_{12}$ aerated with 2–5 % CO_2 in air; 1 month after inoculation .

Fig. 136. Isolate Var I; 1.5 % $BBMPTB_{12}$ agar; 2 months after inoculation.


h
131
100
h
132
100
b
r
133
100
134
10
h
135
10
w
136
10

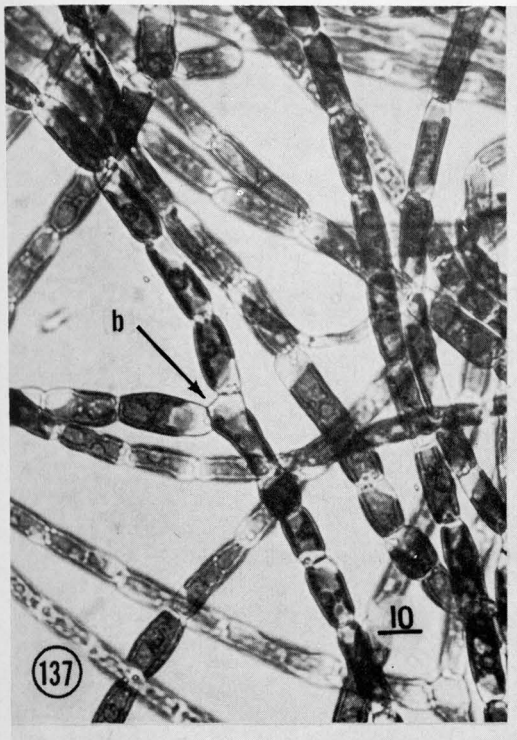
FIGURES 137–140

***Stigeoclonium tenue* (Ag.) Kütz. (Emend.)**
(Size scale in microns)

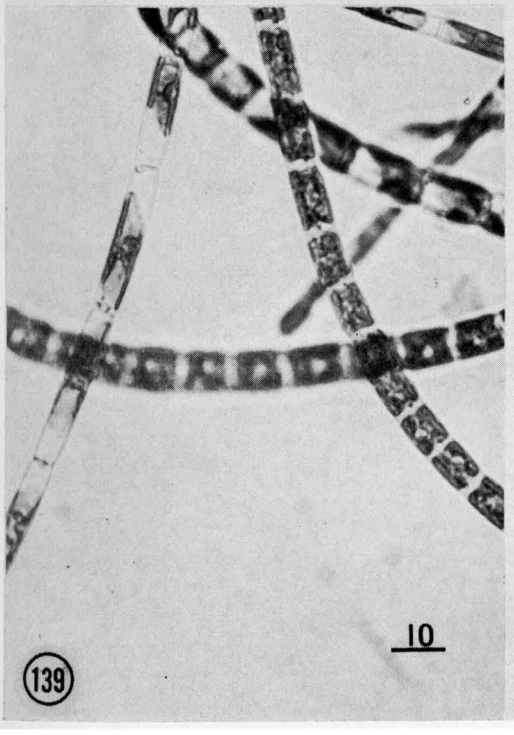
Fig. 137–140. Erect system—four pictures from a single mount. Note that cell shape is extremely variable—both cylindrical without constrictions at partition wall, and barrel-shaped with deep constrictions—and that cell size, especially length, varies also; alternate branching (b).

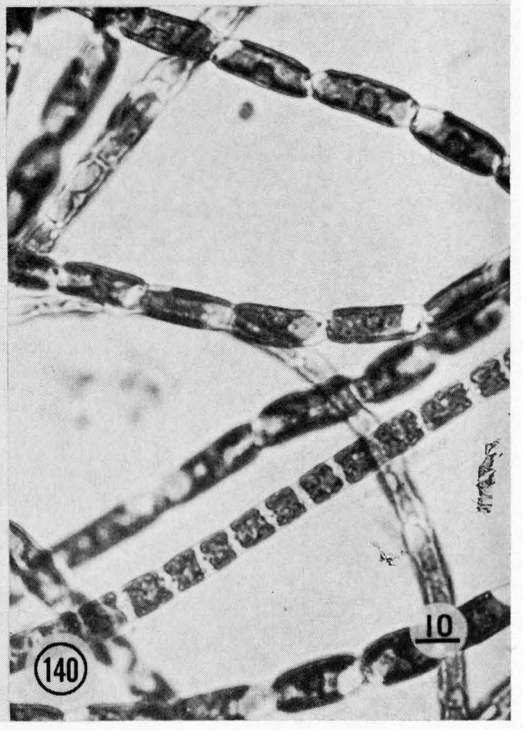
Conditions of culture

Fig. 137–140. Isolate Var I; BBMPTB$_{12}$ aerated with 2–5 % CO_2 in air; 3 weeks after inoculation.


b
10
137


b
10
138


10
139


10
140

FIGURES 141–145

***Stigeoclonium tenue* (Ag.) Kütz. (Emend.)**

Fig. 141. Cylindrical cells of actively growing erect filaments; recent intercalary division (arrows). (Size scale in microns.)

Fig. 142. Cylindrical cells of actively growing erect filaments; blunt tip of branch (t). (Size scale in microns.)

Fig. 143–144. Thick-walled (w), much branched, akinete-like cells; terminal cells of filament often swollen. (Size scale in microns.)

Fig. 145. Caespitose or matted colony on agar (× 24).

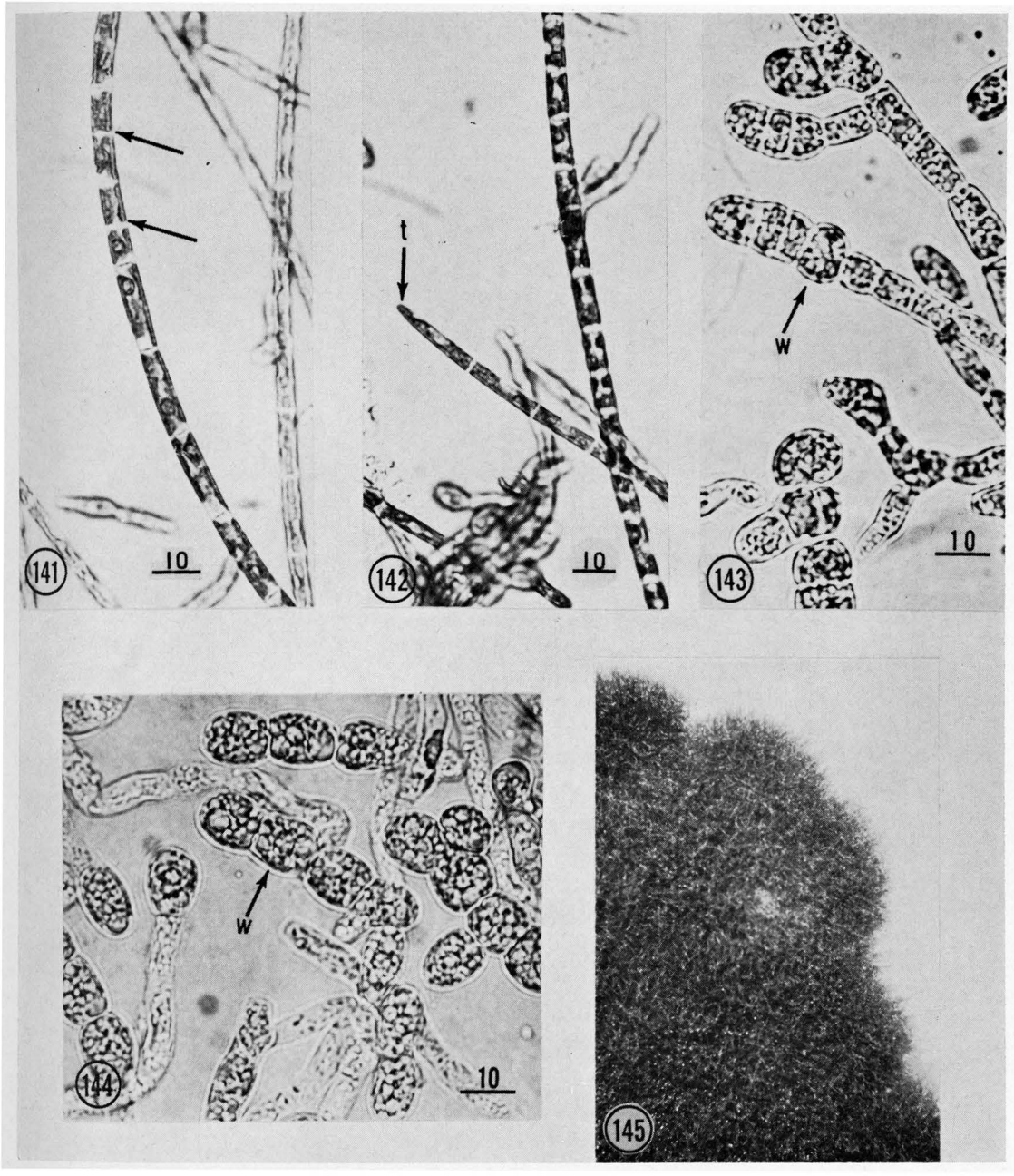
Conditions of culture

Fig. 141–142. Isolate 6–1D; $BBMPTB_{12}$ aerated wtih 2–5 % CO_2 in air; 2 weeks after inoculation.

Fig. 143. Isolate Gold; 1.5 % $BBMPTB_{12}$ agar; 2 months after inoculation.

Fig. 144. Isolate 19–1–E; 1.5 % $BBMPTB_{12}$ agar; 2 months after inoculation.

Fig. 145. Isolate 19–1–E; 1.5 % $BBMPTB_{12}$ agar; 2 months after inoculation.


141
10
142
t
10
143
w
10
144
w
10
145

FIGURES 146–152

***Stigeoclonium pascheri* Vischer comb. nov.**
(Size scale in microns)

Fig. 146. Early germling stage. Development of erect filament (e) from zoospore (z); unilateral development of the prostrate filament (pr).

Fig. 147. Early germling stage. Development of prostrate filaments (pr) and lateral branches (l).

Fig. 148. Bilateral development of basal filament from which prostrate lateral branches (l) and erect filaments (e) arise.

Fig. 149, 150. Alternate branches arising from upper portion of long cell near the septum (b); dichotomous (or alternate?) branching from small cell of main axis (sc); blunt to slightly pointed branch tips (t); intercalary division of cells of main axis (arrows).

Fig. 151. Extensive filamentous prostrate thallus; lateral branches (l) with slightly rhizoidal tips (r); short erect filaments (e).

Fig. 152. Several prostrate thalli.

Conditions of culture

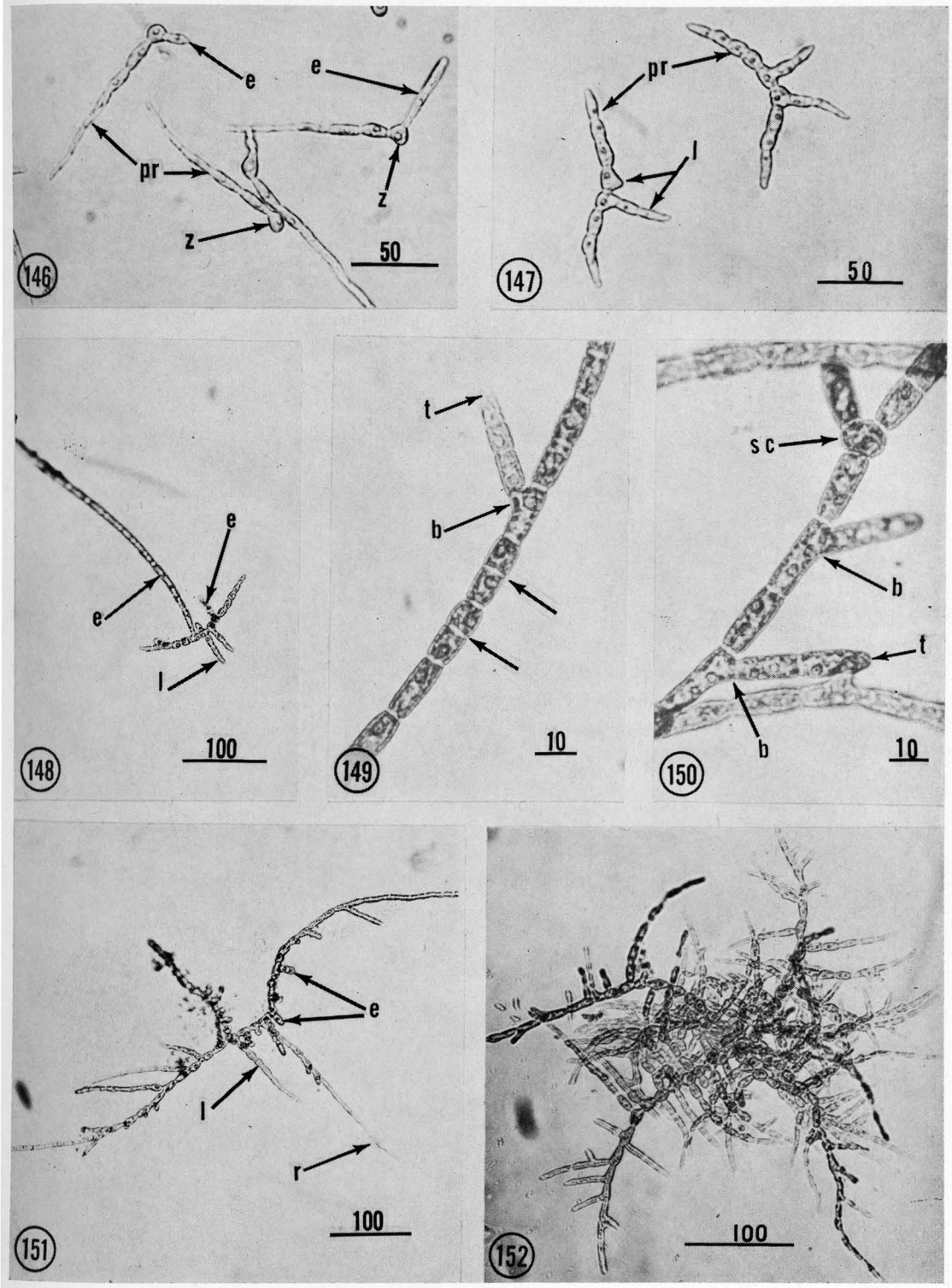
Fig. 146. Isolate 10–2; BBMPTB$_{12}$; 4 days after inoculation.

Fig. 147. Isolate Ca 421; BBMPTB$_{12}$; 4 days after inoculation.

Fig. 148. Isolate 10–2; BBMPTB$_{12}$ aerated with 2–5 % CO_2 in air; 1 week after inoculation.

Fig. 149–151. Isolate 10–2; BBMPTB$_{12}$ aerated with 2–5 % CO_2 in air; 2 weeks after inoculation.

Fig. 152. Isolate 18–3; BBMPTB$_{12}$ aerated with 2–5 % CO_2 in air; 2 weeks after inoculation.


e
e
pr
z
z
50
146
pr
l
50
147
e
e
l
100
148
t
b
10
149
sc
b
t
b
10
150
e
l
r
100
151
100
152

FIGURES 153–157

***Stigeoclonium pascheri* comb. nov.**
(Size scale in microns)

Fig. 153–155. Spreading filamentous prostrate thallus with extensively rebranched lateral filaments.

Fig. 156. Portion of discrete colony in aerated culture illustrating upward proliferation of basal filaments (cf. Fig. 161).

Fig. 157. Globular cells of prostrate thallus (g).

Conditions of culture

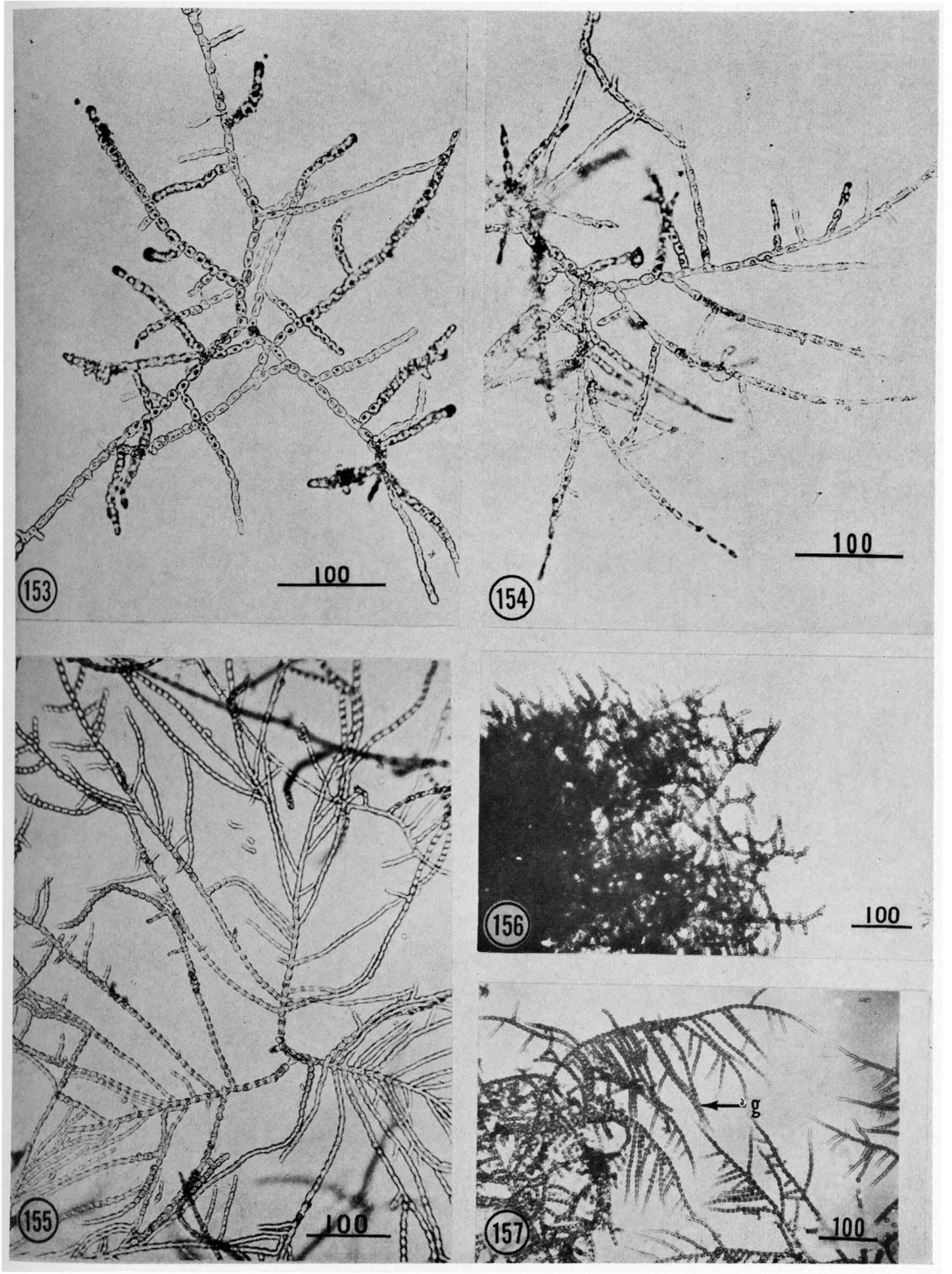
Fig. 153. Isolate 18–3; BBMPTB$_{12}$; 1 week after inoculation.

Fig. 154. Isolate 10–2; BBMPTB$_{12}$; 1 week after inoculation.

Fig. 155. Isolate Ca 421; BBMPTB$_{12}$; 2 weeks after inoculation.

Fig. 156. Isolate 18–3; BBMPTB$_{12}$ aerated with 2–5% CO_2 in air; 1 month after inoculation.

Fig. 157. Isolate Ca 421; BBMPTB$_{12}$; 1 month after inoculation.


153
100
154
100
155
100
156
100
157
g
100

FIGURES 158–163

***Stigeoclonium pascheri* comb. nov.**
(Size scale in microns)

Fig. 158. Globular cells (g) of spreading filamentous prostrate thallus; lateral branches (l) with slightly rhizoidal tips (r).

Fig. 159. Spreading filamentous prostrate thallus with extensive lateral branching.

Fig. 160. Globular cells of prostrate thalli (g).

Fig. 161. Upward proliferation of basal filaments; globular cells of prostrate thalli (g) (cf. Fig. 156).

Fig. 162. Unbranched erect filaments (e); cells of basal filaments (pr).

Fig. 163. Alternately branched erect filaments (e); unilaterally branched prostrate filament resulting from *in situ* germination of zoospores (pr); portion of empty filament after zoospore release (zr).

Conditions of culture

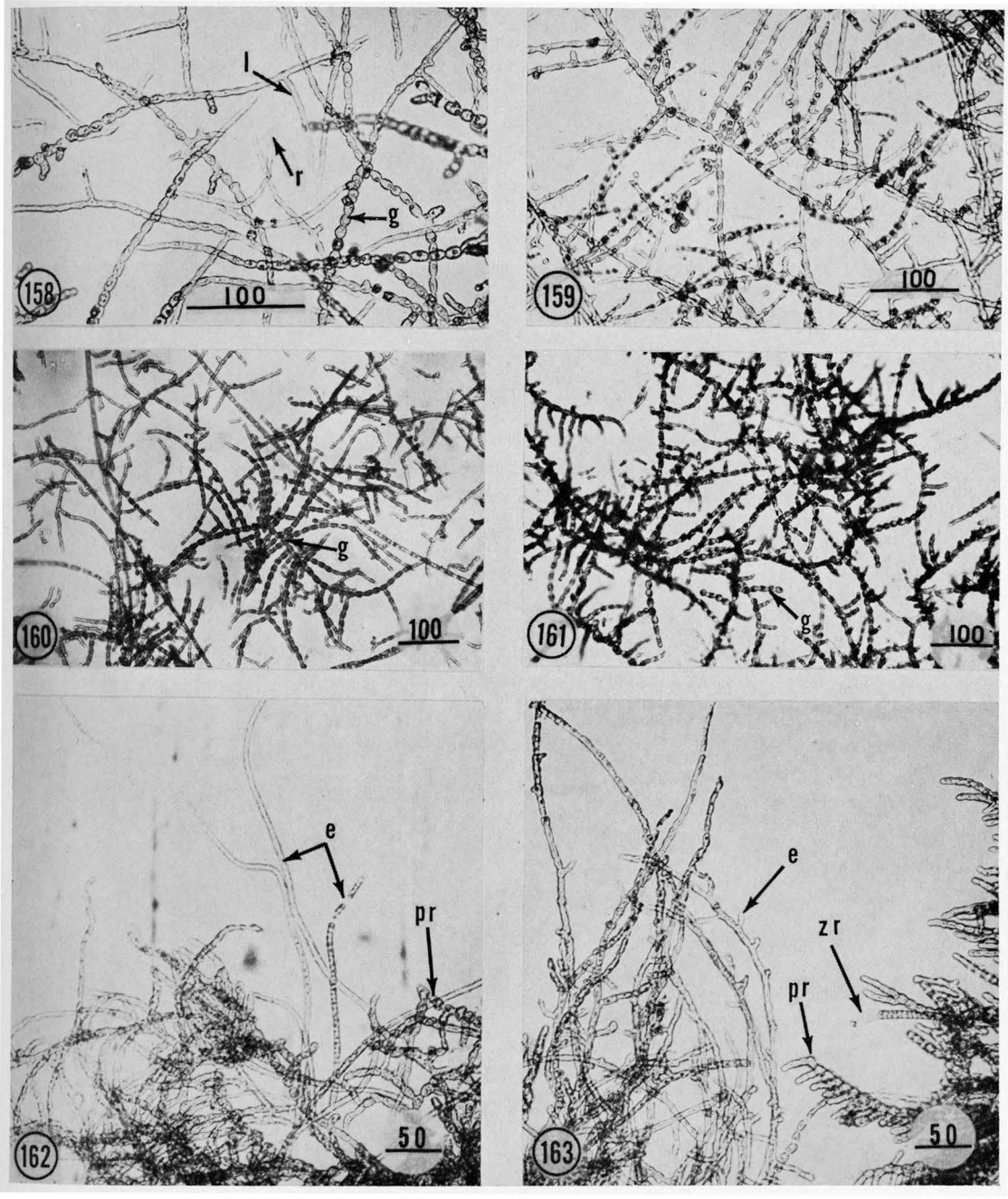
Fig. 158. Isolate 18–3; $BBMPTB_{12}$; 3 weeks after inoculation.

Fig. 159. Isolate 18–3; $BBMPTB_{12}$; 2 weeks after inoculation.

Fig. 160. Isolate 10–2; $BBMPTB_{12}$; 1 month after inoculation.

Fig. 161. Isolate 18–3; $BBMPTB_{12}$; 1 month after inoculation.

Fig. 162, 163. Isolate 18–3; $BBMPTB_{12}$ aerated with 2–5 % CO_2 in air; 3 weeks after inoculation.


158
l
r
g
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159
100
160
g
100
161
g
100
162
e
pr
50
163
e
zr
pr
50

FIGURES 164–168

***Stigeoclonium pascheri* comb. nov.**

Fig. 164. Thick-walled (w), globose akinetes. (Size scale in microns.)

Fig. 165. Much-branched filaments; thick-walled (w) globose akinetes; bulbous cells at end of filaments (c). (Size scale in microns.)

Fig. 166, 167. Thick-walled (w), globose akinetes; partition of cell contents into 2 (Fig. 166, 167) or 4 parts (Fig. 167). (Size scale in microns.)

Fig. 168. Caespitose or matted colony on agar (× 24).

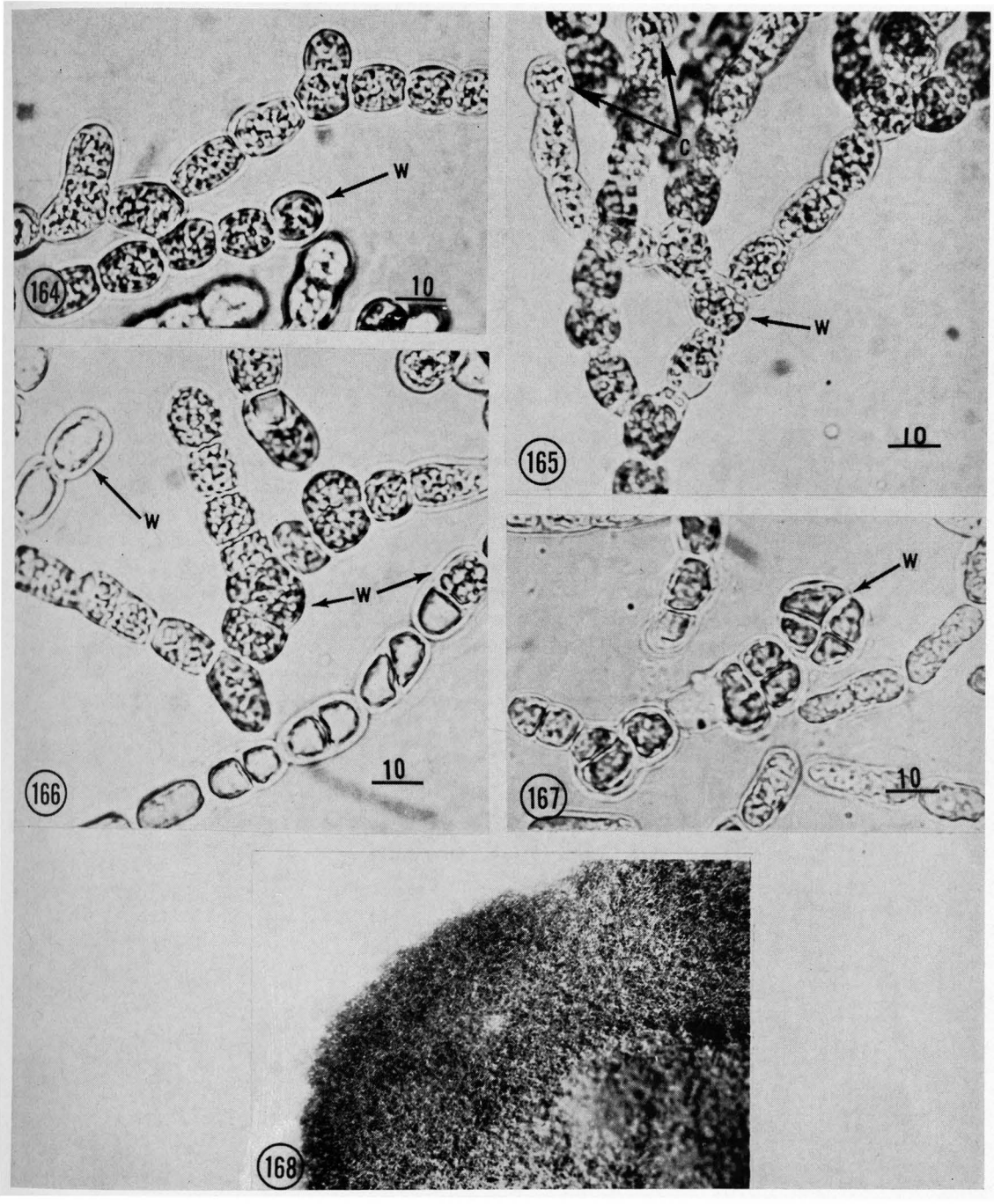
Conditions of culture

Fig. 164. Isolate Ca 421; 1.5 % $BBMPTB_{12}$ agar; 2 months after inoculation.

Fig. 165. Isolate 18–3; 1.5 % $BBMPTB_{12}$ agar; 2 months after inoculation.

Fig. 166, 167. Isolate 10–2; 1.5 % $BBMPTB_{12}$ agar; 2 months after inoculation.

Fig. 168. Isolate Ca 421; 1.5 % $BBMPTB_{12}$ agar; 1 month after inoculation.


W
164
10
C
W
165
10
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W
166
10
W
167
10
168

FIGURES 169–176

Stigeoclonium variabile (Nageli) Islam

Fig. 169–170. Early germling stage. Germinating zoospore (z); equal bilateral development of primary basal filament; development of lateral prostrate branches (l) and erect filaments (e) from primary basal filment.

Fig. 171, 176. Equal bilateral development of primary basal filament; formation of rebranching prostrate lateral filaments (l) and erect filaments (e).

Fig. 172. Bilateral development of primary basal filament; rebranching prostrate lateral filaments with slightly rhizoidal terminal cells (r); globular cells of basal system (g); type-I germination (arrow).

Fig. 173–174. Predominant unilateral development (Fig. 173) or bilateral development (Fig. 174) of primary basal filament; formation of prostrate lateral branches and erect filaments from primary basal filament; type-I germination (Fig. 173—arrow).

Fig. 175. Small branching filamentous basal system; formation of long, unbranched erect filaments (e) from cells of basal system.

Conditions of culture

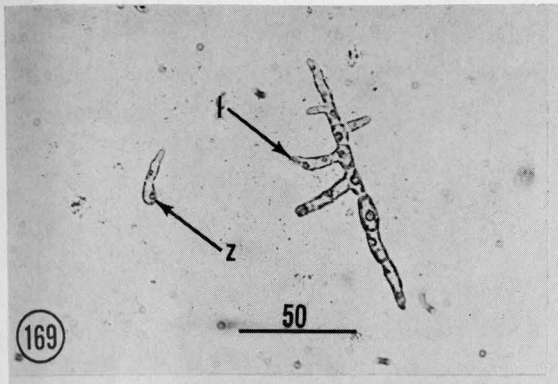
Fig. 169, 170. Isolate Jo; $BBMPTB_{12}$; 1 week after inoculation.

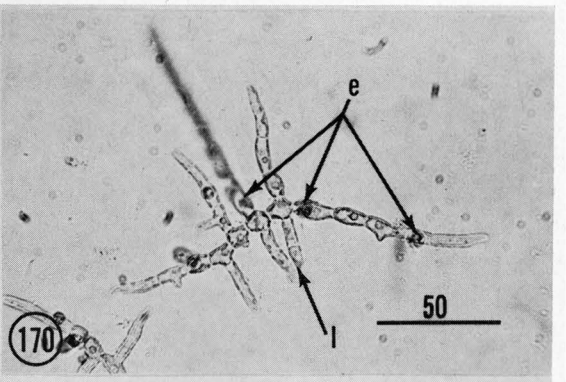
Fig. 171. Isolate 6–23; $BBMPTB_{12}$; 1 week after inoculation.

Fig. 172. Isolate 6–15; $BBMPTB_{12}$; 2 weeks after inoculation.

Fig. 173–175. Isolate Jo; $BBMPTB_{12}$; 2 weeks after inoculation.

Fig. 176. Isolate 6–23; $BBMPTB_{12}$ aerated with 2–5 % CO_2 in air; 2 weeks after inoculation.


l
z
50
169


e
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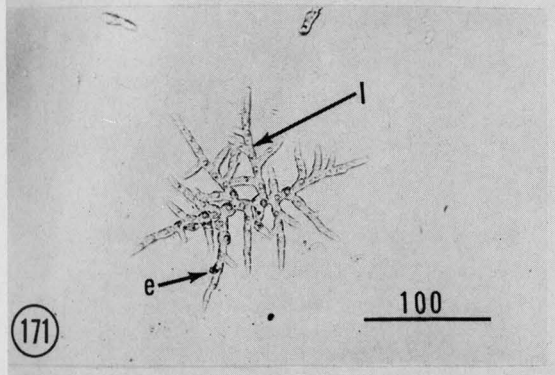
170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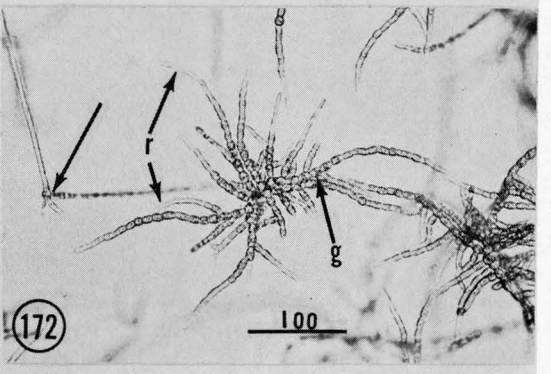
171


r
g
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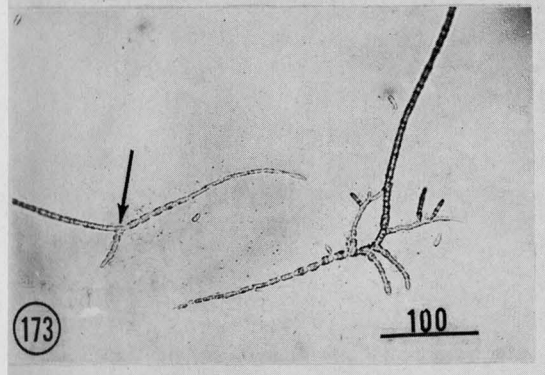
172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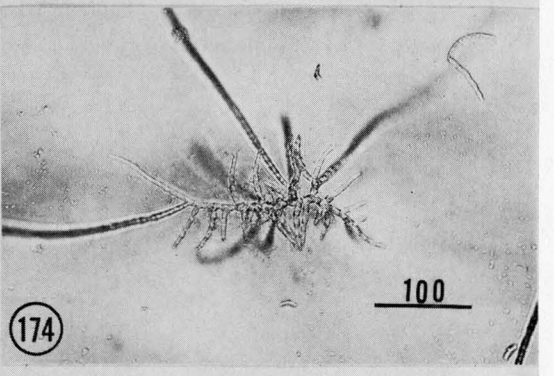
173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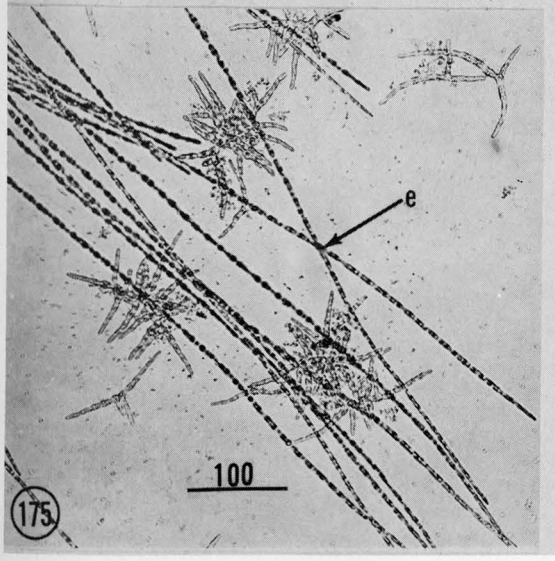
e
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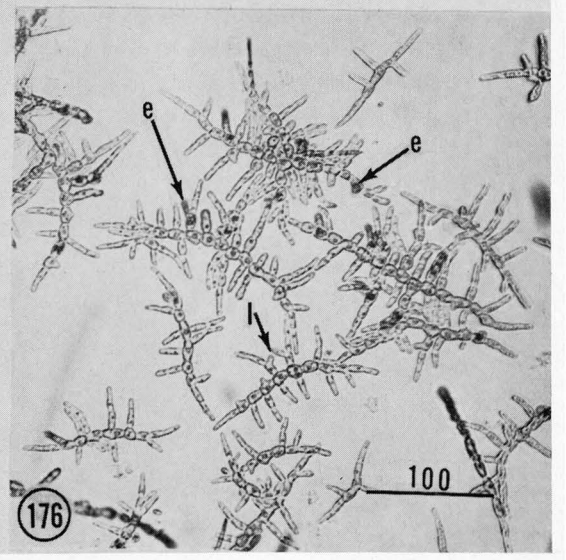
175


e
e
l
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176

FIGURES 177–185

***Stigeoclonium variabile* (Nageli) Islam**
(Size scale in microns)

Fig. 177. Small branching filamentous basal system; erect filaments terminating in multicellular colorless hairs (h); zoospores (z).

Fig. 178–180. Formation of erect filaments (e) and prostrate lateral branches (l) from cells of basal system; rebranching of the prostrate lateral branches shown clearly in Fig. 180.

Fig. 181. Prostrate thallus near surface of culture medium—at evaporation level. Note the arrested development of the lateral branches of the basal system and the formation of globular, akinete-like cells (g).

Fig. 182, 185. Compact basal system, illustrating later stages of Fig. 183 and 184.

Fig. 183–184. Formation of rebranching lateral prostrate filaments (l) and erect filaments (e) from primary basal filament; Fig. 183—unbranched erect filaments (e) terminating in *long*, multicellular colorless hairs (h).

Conditions of culture

Fig. 177. Isolate 6–23; BBMPTB$_{12}$ aerated with 2–5 % CO_2 in air; 2 weeks after inoculation.

Fig. 178. Isolate Jo; BBMPTB$_{12}$; 3 weeks after inoculation.

Fig. 179. Isolate 6–23; BBMPTB$_{12}$; 3 weeks after inoculation.

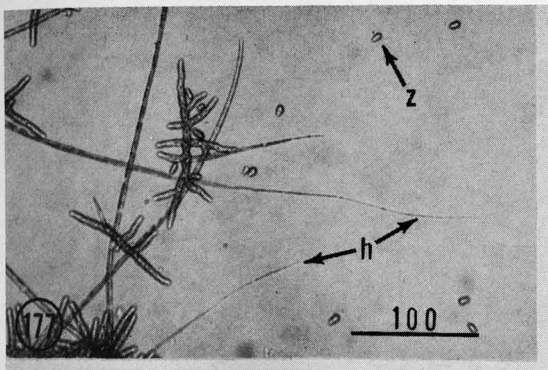
Fig. 180–181. Isolate 6–15; BBMPTB$_{12}$; 1 month after inoculation.

Fig. 182. Isolate 6–23; 3 BBMP:1 SS; 1 month after inoculation.

Fig. 183. Isolate 6–15; 3 BBMP:1 SS; 2 weeks after inoculation.

Fig. 184. Isolate 6–15; BBMPTB$_{12}$; 1 week after inoculation.

Fig. 185. Isolate 6–15; 3 BBMP:1 SS; 1 month after inoculation.


z
h
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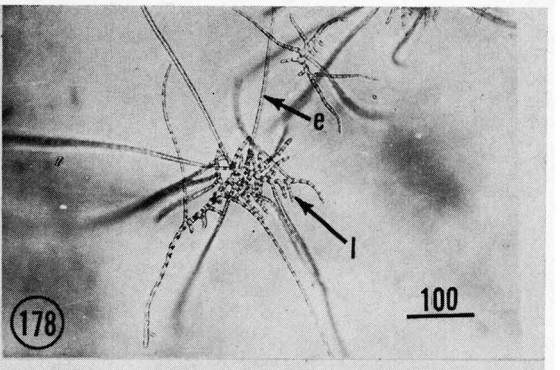
177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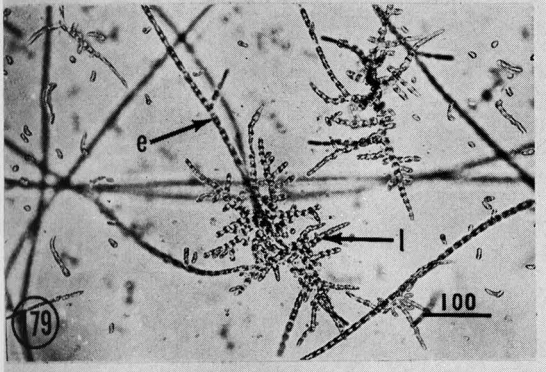
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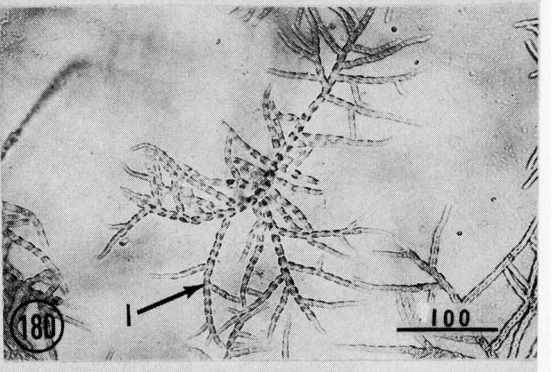
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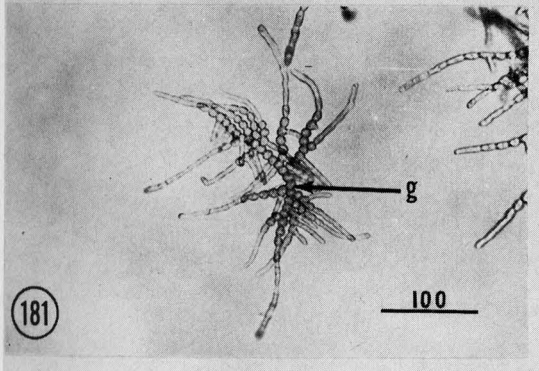
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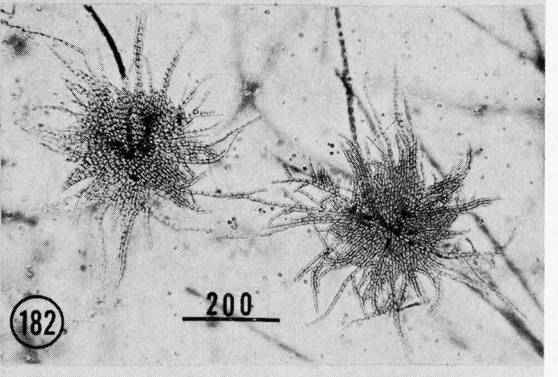
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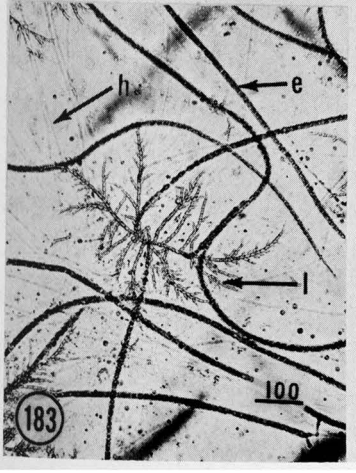
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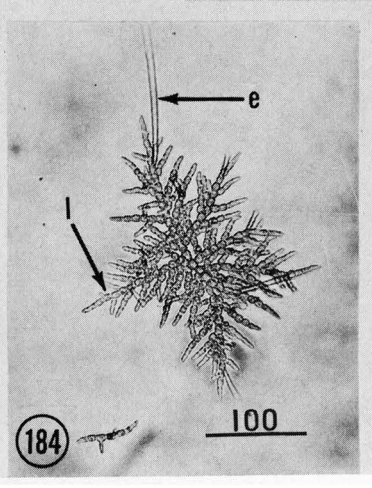
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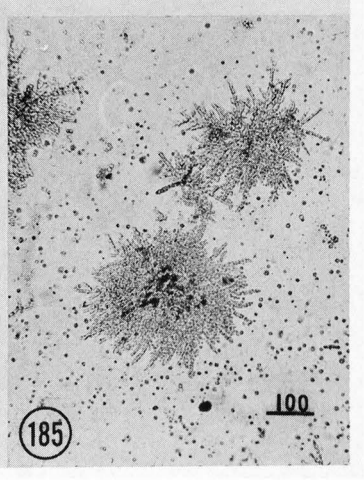
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185

FIGURES 186–189

***Stigeoclonium variabile* (Nageli) Islam**
(Size scale in microns)

Fig. 186. Basal system with rebranched lateral prostrate filaments (l); extensive erect system developed from cells of basal system; cells of erect system cylindrical; alternate branching; branch tips terminating in acute points (t) or multicellular colorless hairs (h); erect filaments *not radiating* from basal system.

Fig. 187–188. Compact basal system; short, unbranched erect filaments (e) *radiating* from cells of basal system; some erect filaments terminating in colorless hairs (h), others with blunt tips (t).

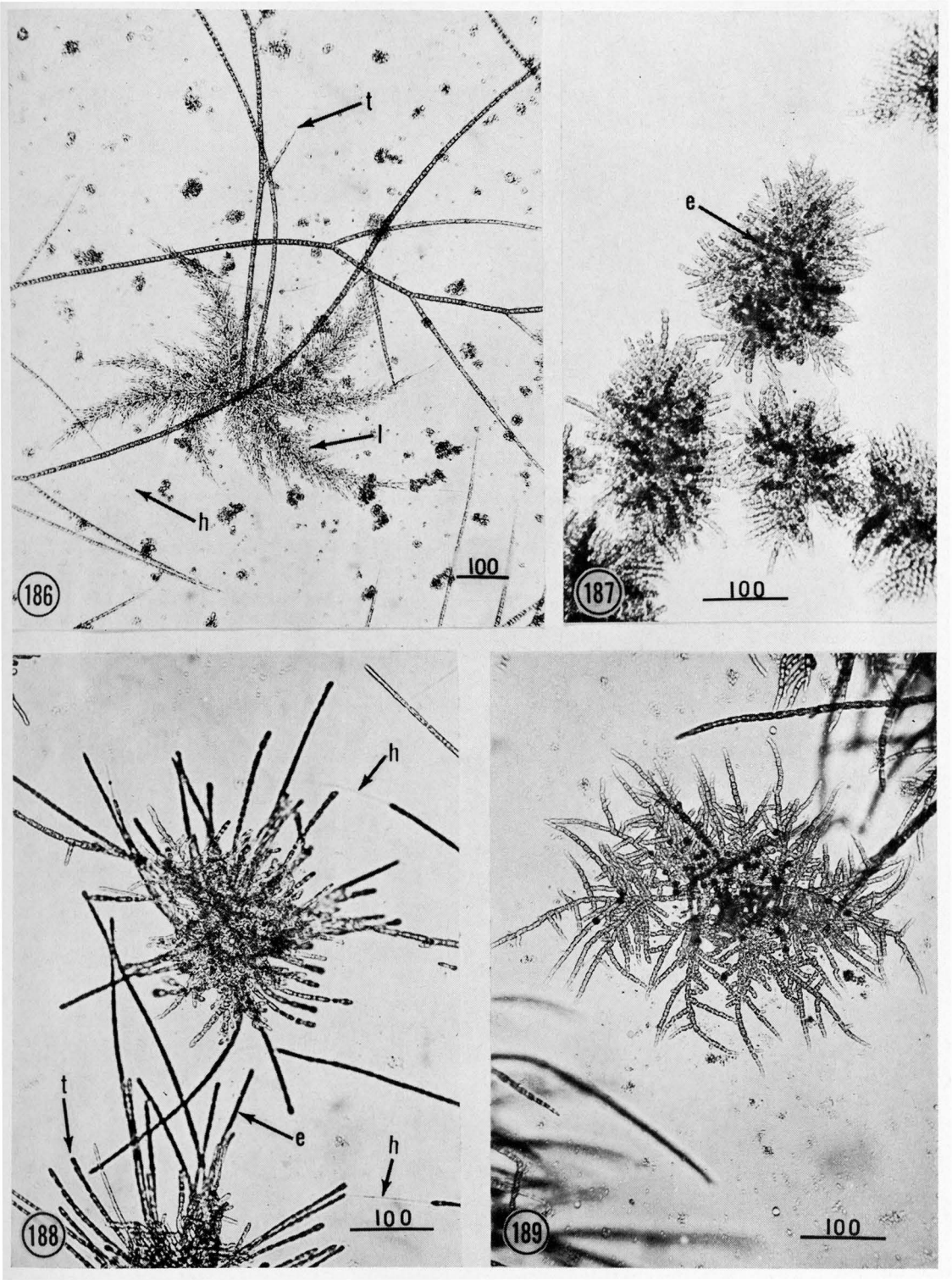
Fig. 189. Basal system. Formation of rebranching lateral prostrate filaments and erect filaments from primary basal filaments.

Conditions of culture

Fig. 186, 189. Isolate 6–15; 3 BBMP:1 SS; 2 weeks after inoculation.

Fig. 187. Isolate 6–23; 1 week $BBMPTB_{12}$ in laboratory, 2 weeks Blanco River at San Marcos, Texas.

Fig. 188. Isolate 6–15; $BBMPTB_{12}$; 2 weeks after inoculation.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e
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189

FIGURES 190–195

***Stigeoclonium variabile* (Nageli) Islam**
(Size scale in microns)

Fig. 190–191. Compact basal system composed of rebranching lateral prostrate filaments; globular, akinete-like cells of basal system; erect filaments (e).

Fig. 192–195. Differences in degree of branching, branching pattern, and nature of branch tip of the erect filaments:

Fig. 192. Alternate (dichotomous ?) branching (b); secund branching; approximate branching (a); branch tip acute (t).

Fig. 193. Secund branching (s); unbranched erect filaments; branch tips blunt (t) or with multicellular colorless hairs (h).

Fig. 194. Unbranched erect filaments; branch tips acute (t) or with multicellular colorless hairs (h).

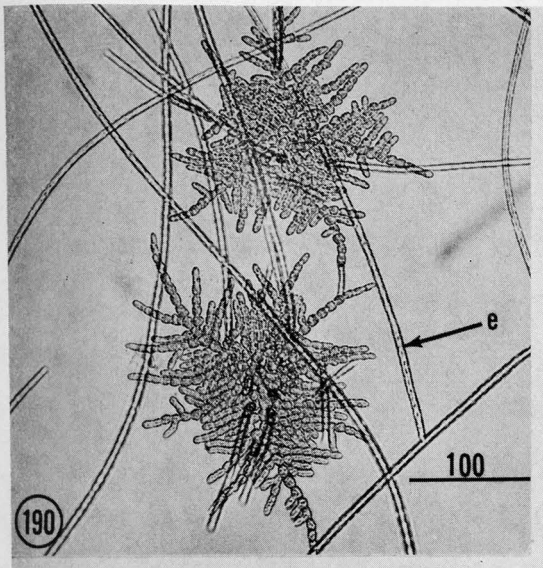
Fig. 195. Erect filaments profusely branched in an irregular, secund manner—probably from fragment of inoculum which failed to release zoospores; branch tip acute (t).

Conditions of culture

Fig. 190. Isolate Jo; $BBMPTB_{12}$; 1 week after inoculation.

Fig. 191. Isolate Jo; 1 week $BBMPTB_{12}$ in laboratory, 2 weeks Blanco River, San Marcos, Texas.

Fig. 192–195. Isolate 6–15; $BBMPTB_{12}$; 2 weeks after inoculation.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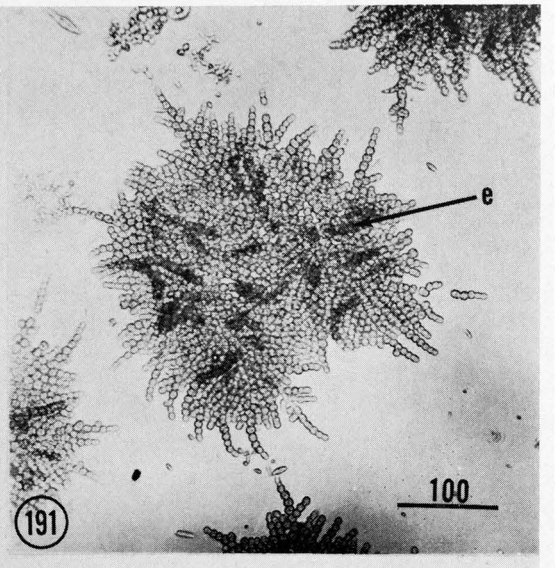
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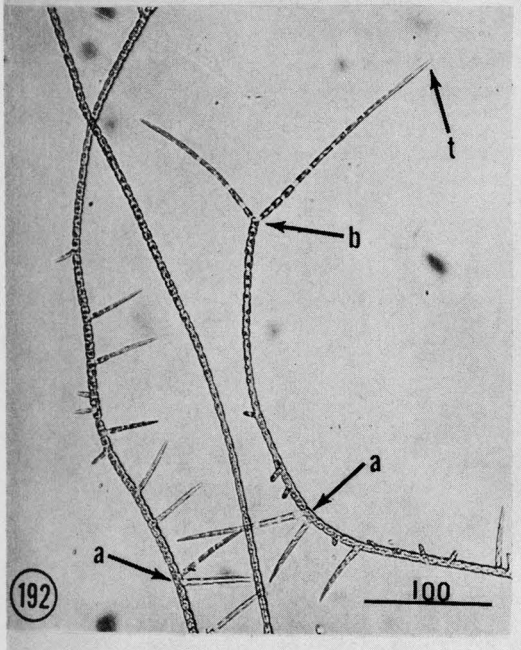
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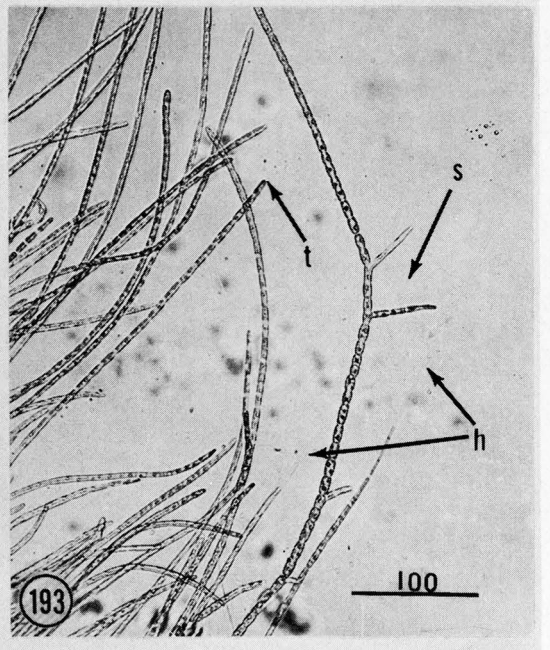
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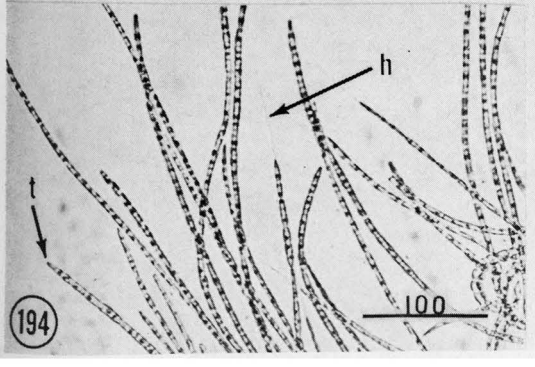
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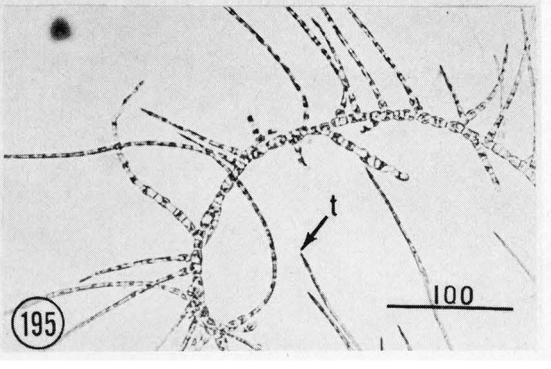
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195

FIGURES 196–202

***Stigeoclonium variabile* (Nageli) Islam**
(Size scale in microns)

Fig. 196–198. Erect system. Cylindrical, little constricted cells; alternate branching (b); approximate branching (a); opposite branching (0—Fig. 196); branch tips either acute or blunt.

Fig. 199, 201. Erect system. Barrel-shaped or constricted cells of main filament; filaments profusely branched, nearly every cell forming a branch (especially Fig. 199); branching unilateral, sometimes bilateral, and approximate; branch tips blunt (t).

Fig. 200. Alternate, unilateral, and approximate branching; branch tips pointed or blunt.

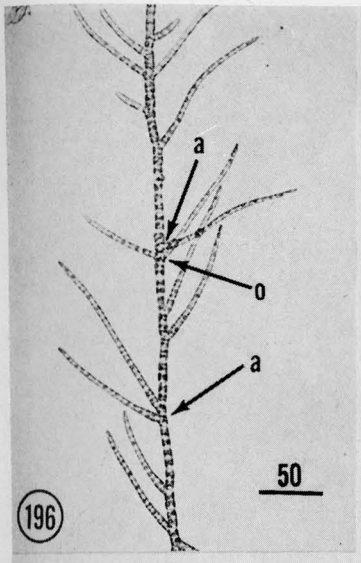
Fig. 202. Unilateral branching; branch tips blunt.

Conditions of culture

Fig. 196–198. Isolate Jo; $BBMPTB_{12}$ aerated with 2–5 % CO_2 in air; 2 weeks after inoculation.

Fig. 199, 200. Isolate Jo; $BBMPTB_{12}$ aerated with 2–5 % CO_2 in air; 3 weeks after inoculation.

Fig. 201, 202. Isolate Jo; $BBMPTB_{12}$; 1 month after inoculation.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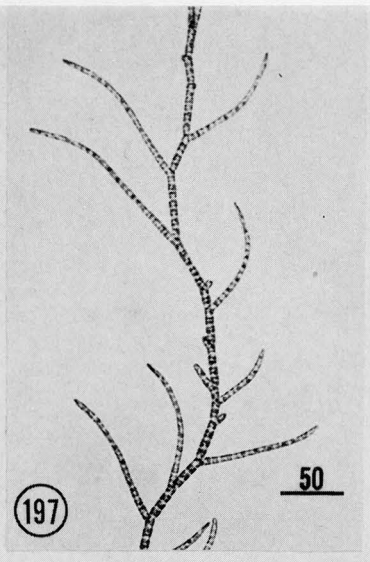
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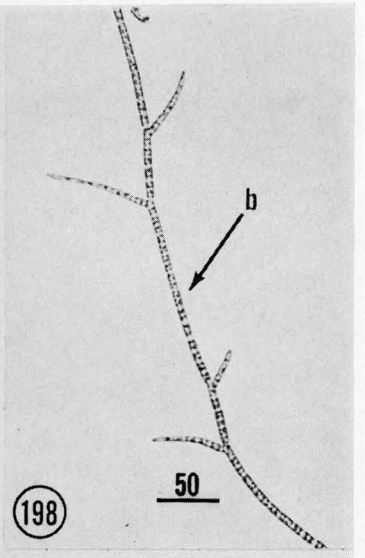
b
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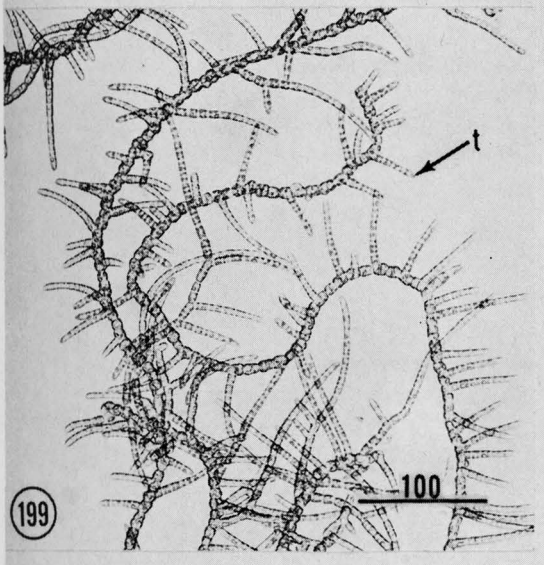
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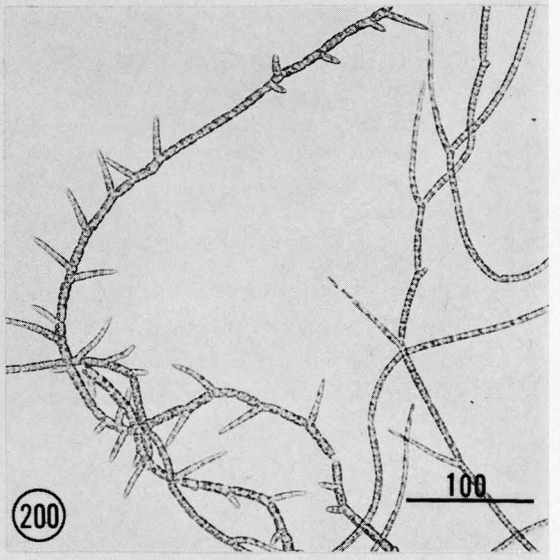
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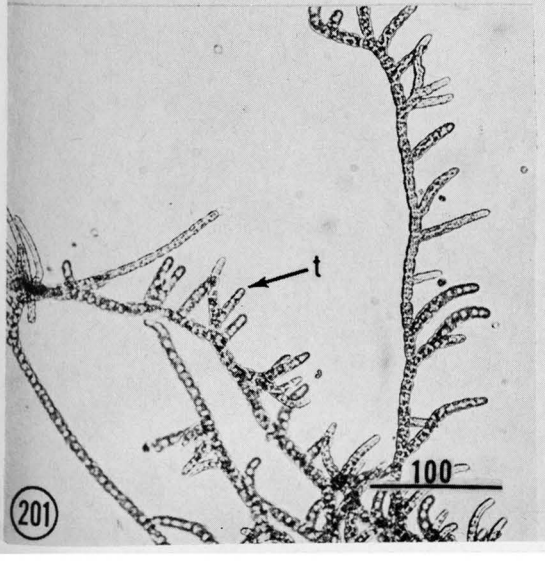
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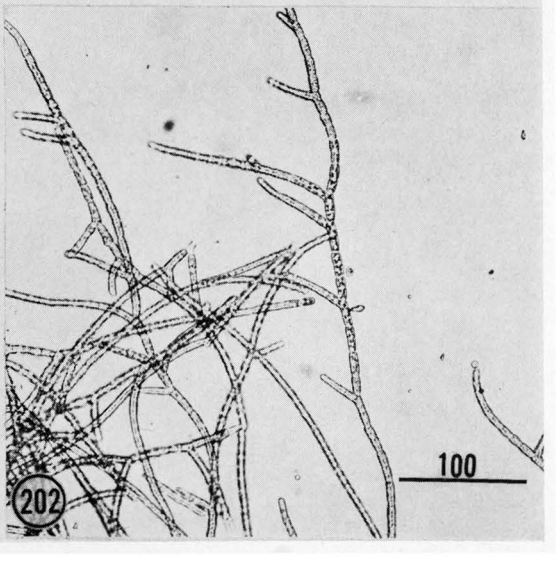

100
202

FIGURES 203–207

***Stigeoclonium variabile* (Nageli) Islam**
(Size scale in microns)

Fig. 203–204. Barrel-shaped or constricted cells of erect filaments (g); branch tips blunt (t); unilateral branching (Fig. 204—from almost every cell of the filament).

Fig. 205–206. Portion of erect system and basal system (bs) from river collection. Short, usually unbranched, erect filaments (e) terminating in long, multicellular colorless hairs (h).

Fig. 207. Much-branched filament; thick-walled (w) akinete-like cells; terminal cells of filament often bulbous (c).

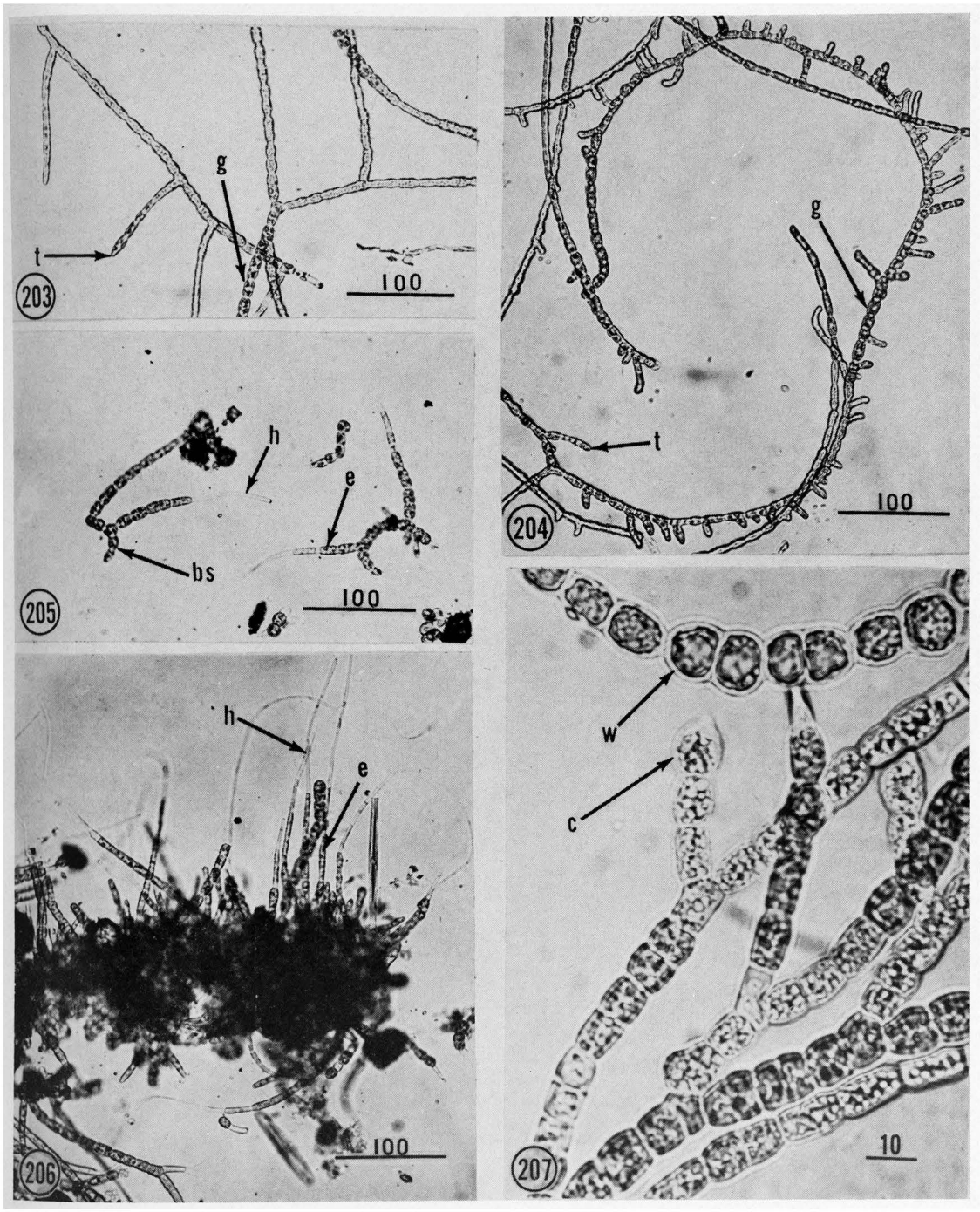
Conditions of culture

Fig. 203. Isolate 6–23; BBMPTB$_{12}$; 1 month after inoculation.

Fig. 204. Isolate 6–15; BBMPTB$_{12}$; 1 month after inoculation.

Fig. 205, 206. Isolate 6–23; 1 week BBMPTB$_{12}$ in laboratory, 2 weeks Blanco River, San Marcos, Texas.

Fig. 207. Isolate Jo; 1.5 % BBMPTB$_{12}$ agar; 2 months after inoculation.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g
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203
g
t
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204
h
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bs
100
205
h
e
100
206
w
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10
207

FIGURES 208–210

***Stigeoclonium variabile* (Nageli) Islam**

Fig. 208–210. ***Schizothrix*-like tufts which characterize colony on agar.**

Fig. 208, 210—× 24.

Fig. 209—× 48.

Conditions of culture

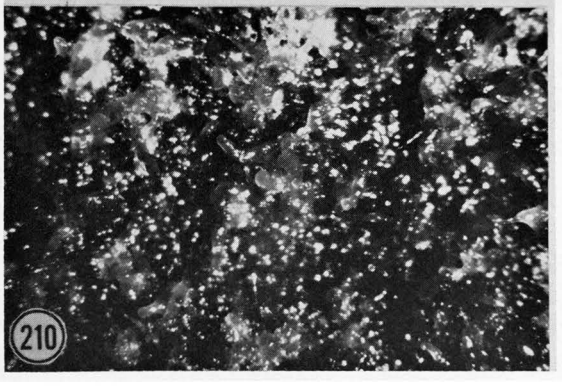
Fig. 208. **Isolate Jo; 1.5% BBMPTB$_{12}$ agar; 1 month after inoculation.**

Fig. 209. **Isolate 6–23; 1.5% BBMPTB$_{12}$ agar; 1 month after inoculation.**

Fig. 210. **Isolate 6–15; 1.5% BBMPTB$_{12}$ agar; 1 month after inoculation.**


208


209


210

FIGURES 211–218

***Stigeoclonium farctum* Berthold**
(Size scale in microns)

Fig. 211. Zoospores come to rest and begin to germinate shortly after release from the erect filaments.

Fig. 212. Zoospore (z); (1) young germling showing bipolar germination and beginning of lateral branch formation; (2) young germling: development of erect filament (e) from zoospore (z) and prostrate filaments with lateral branches from lower cell of erect filament—i.e., the zoospore.

Fig. 213–217. Young germling: development of prostrate lateral branches (l) and erect filaments (e) from primary basal filament.

Fig. 218. Proliferation of lateral prostrate filaments to form pseudoparenchymatous disc-like basal system.

Conditions of culture

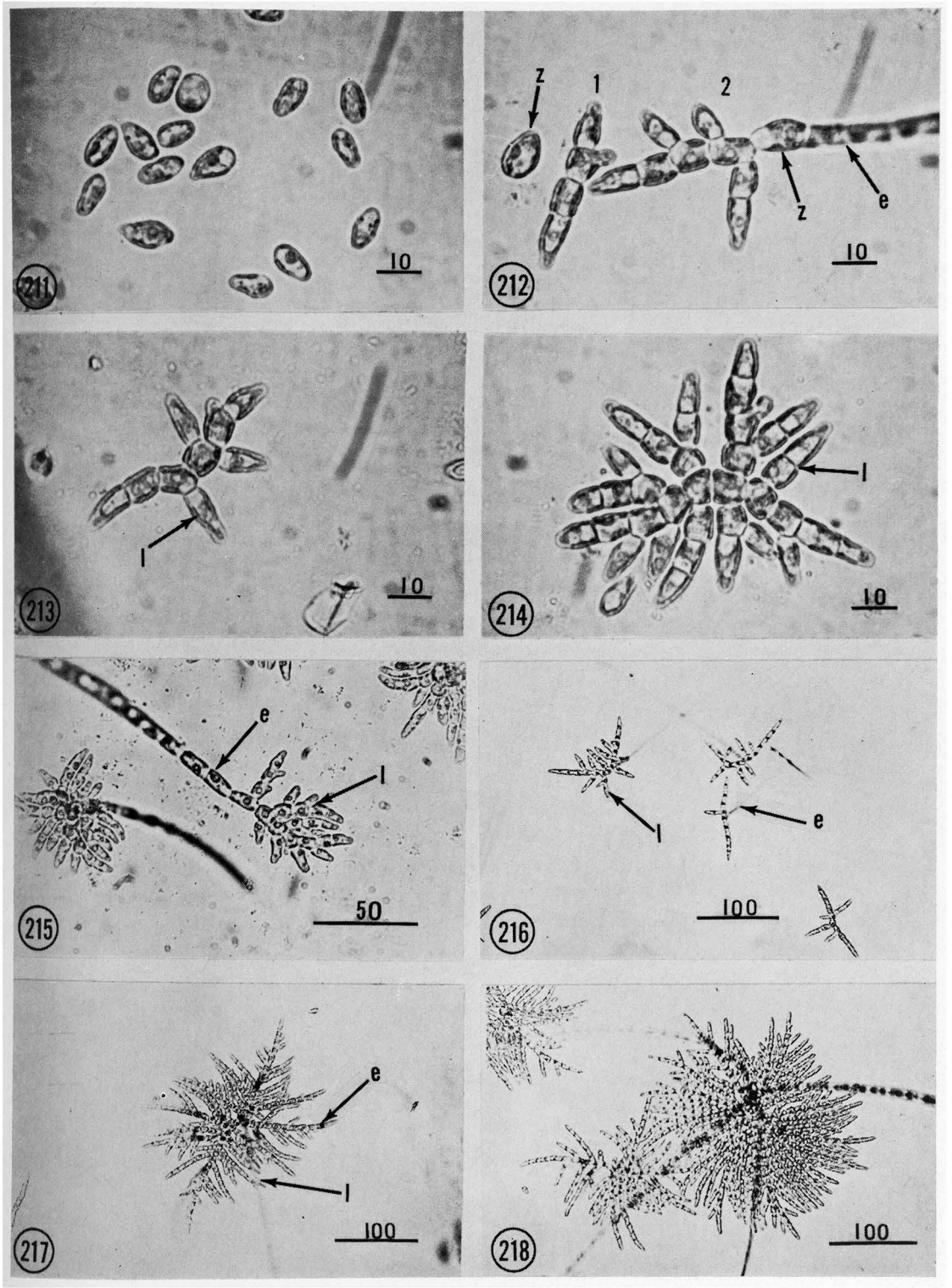
Fig. 211–213. Isolate 5–3F; $BBMPTB_{12}$ aerated with 2–5 % CO_2 in air; 2 weeks after inoculation.

Fig. 214. Isolate 5–3C; $BBMPTB_{12}$ aerated with 2–5 % CO_2 in air; 2 weeks after inoculation.

Fig. 215. Isolate 5–3C; $BBMPTB_{12}$; 5 days after inoculation.

Fig. 216–217. Isolate 7–17; $BBMPTB_{12}$; 1 week after inoculation.

Fig. 218. Isolate 7–17; $BBMPTB_{12}$; 2 weeks after inoculation.


211
10
212
z
1
2
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e
10
213
l
10
214
l
10
215
e
l
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216
l
e
100
217
e
l
100
218
100

FIGURES 219–226

Stigeoclonium farctum **Berthold**
(Size scale in microns)

Fig. 219. Several young germlings. Rhizoidal tip (r) which developed from end of fragment of inoculum which failed to produce zoospores.

Fig. 220. Proliferation of lateral prostrate filaments (l); unbranched erect filaments (e).

Fig. 221–226. Typical pseudoparenchymatous disc-like basal system.

Fig. 223–226. Unbranched erect filaments.

Fig. 226. Alternately branched erect filaments.

Fig. 224–225. Multicellular, colorless hairs (h) at branch tips.

Fig. 226. Tips of branches acute or pointed (t).

Conditions of culture

Fig. 219. Isolate 5–3F; BBMPTB$_{12}$; 2 weeks after inoculation.

Fig. 220. Isolate 7–17; BBMPTB$_{12}$; 3 weeks after inoculation.

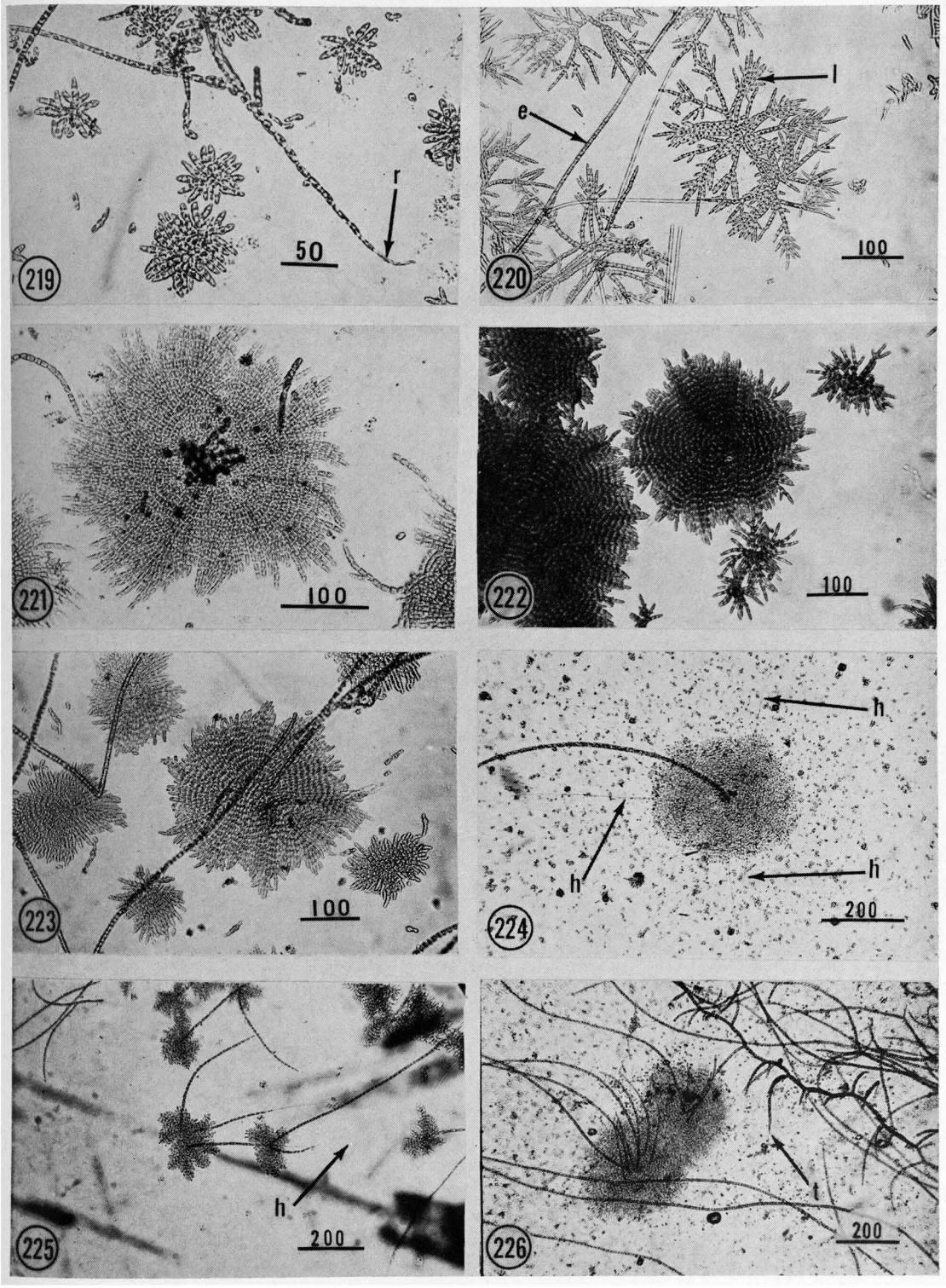
Fig. 221. Isolate 5–3C; BBMPTB$_{12}$; 2 weeks after inoculation.

Fig. 222. Isolate 7–17; BBMPTB$_{12}$ aerated wtih 2–5 % CO_2 in air; 1 month after inoculation.

Fig. 223. Isolate 5–3F; BBMPTB$_{12}$; 3 weeks after inoculation.

Fig. 224. Isolate 7–17; 3 BBMP:1 SS; 2 weeks after inoculation.

Fig. 225–226. Isolate 19–5–V; 3 BBMP:1 SS; 2 weeks after inoculation.


219
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224
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t
200

FIGURES 227–232

***Stigeoclonium farctum* Berthold**
(Size scale in microns)

Fig. 227–230. Typical pseudoparenchymatous disc-like basal system.

Fig. 227. Erect filaments (e).

Fig. 228. Profuse secund branching of erect filaments; edges of disc detaching from substratum (arrow).

Fig. 231–232. Cells of erect filaments slightly barrel-shaped; secund, and approximate (a) branching; branch tips blunt (t).

Conditions of culture

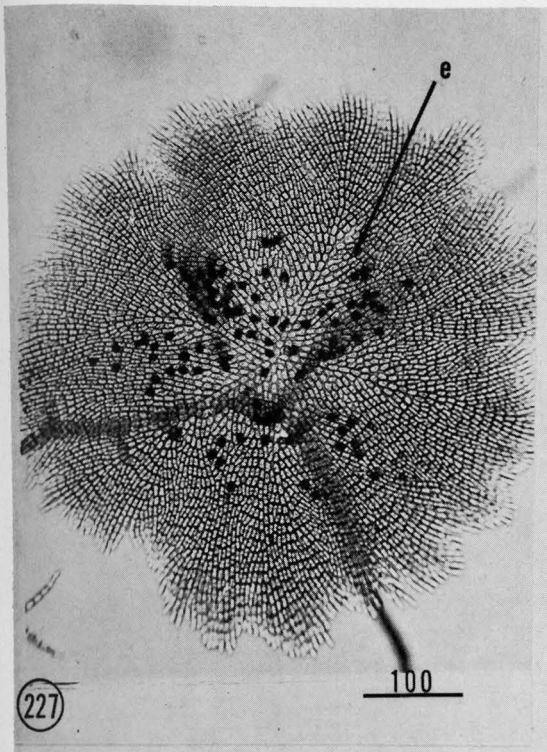
Fig. 227. Isolate 19–5–V; BBMPTB$_{12}$; 2 weeks after inoculation.

Fig. 228. Isolate 19–5–V; 3 BBMP:1 SS; 1 month after inoculation.

Fig. 229. Isolate 5–3C; 1 week BBMPTB$_{12}$ in the laboratory, 2 weeks Blanco River, San Marcos, Texas.

Fig. 230. Isolate 7–17; 1 week BBMPTB$_{12}$ in the laboratory, 2 weeks Blanco River, San Marcos, Texas.

Fig. 231–232. Isolate 5–3C; BBMPTB$_{12}$; 1 month after inoculation.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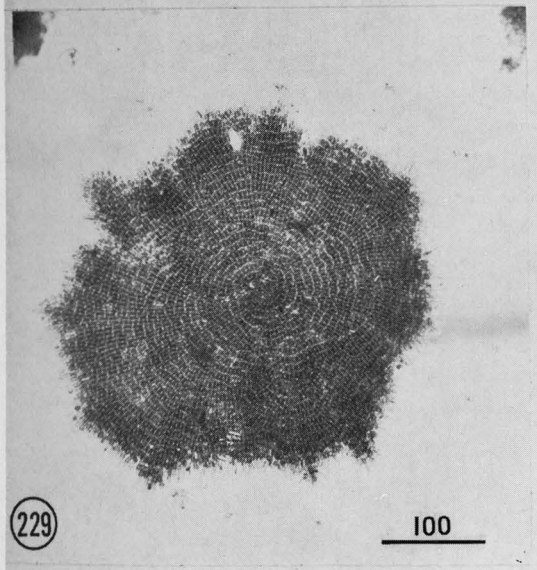
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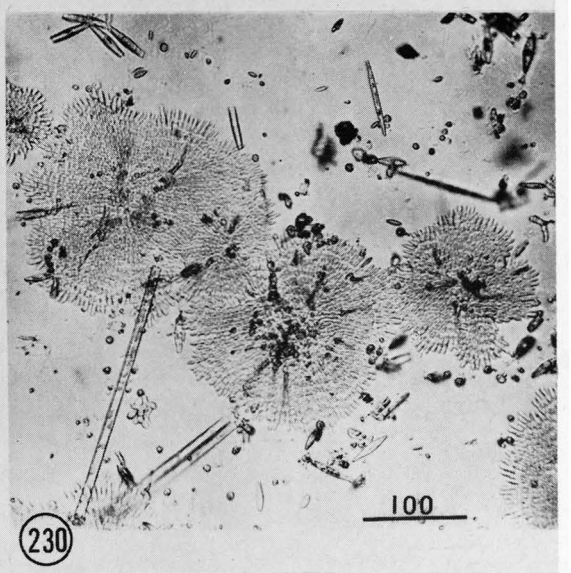
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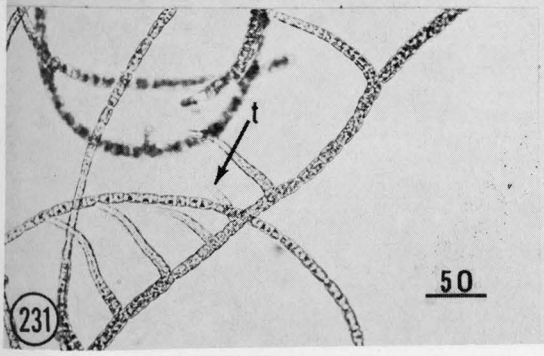
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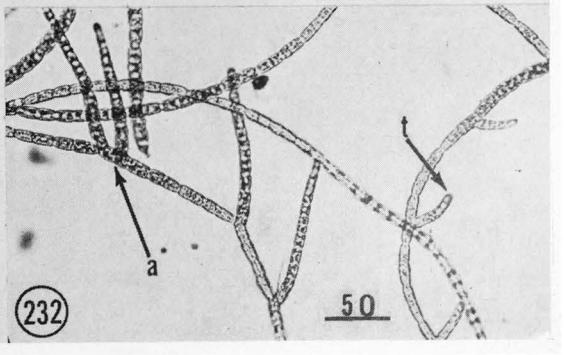
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232

FIGURES 223–239

***Stigeoclonium farctum* Berthold**
(Size scale in microns)

Fig. 233–235, 239. Differences in shape of cells of erect system; branching pattern, and branch tips:
Cells cylindrical—Fig. 233, 234.
Cells barrel-shaped or constricted—Fig. 235, 239 (g).
Secund branching—Fig. 233, 239.
Dichotomous branching—Fig. 234.
Alternate branching—Fig. 235, 239 (a).
Blunt branch tips—Fig. 233 (t).
Acute or pointed branch tips—Fig. 239 (t).
Tips with multicellular colorless hairs—Fig. 235 (h).

Fig. 236. Unbranched erect filaments with cylindrical cells; branch tips blunt.

Fig. 237–238. Thick-walled (w), akinete-like cells; division of cell contents into 2–3 parts (Fig. 237); bulbous cells (c) at terminal end of filament (Fig. 238).

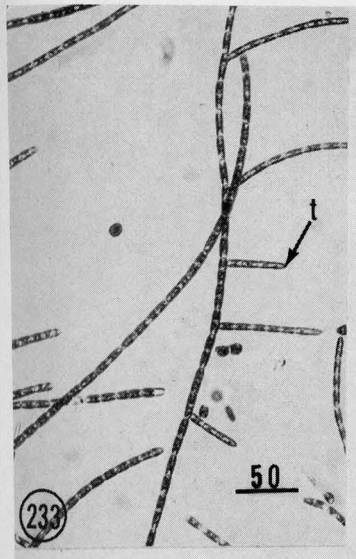
Conditions of culture

Fig. 233–235, 239. Isolate 7–17; BBMPTB$_{12}$ aerated with 2–5 % CO_2 in air; 1 month after inoculation.

Fig. 236. Isolate 5–3C; BBMPTB$_{12}$ aerated with 2–5 % CO_2 in air; 2 weeks after inoculation.

Fig. 237. Isolate 5–3C; 1.5 % BBMPTB$_{12}$ agar; 2 months after inoculation.

Fig. 238. Isolate 19–5–V; 1.5 % BBMPTB$_{12}$ agar; 2 months after inoculation.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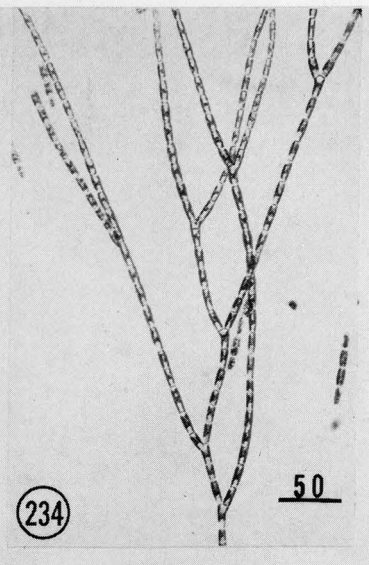
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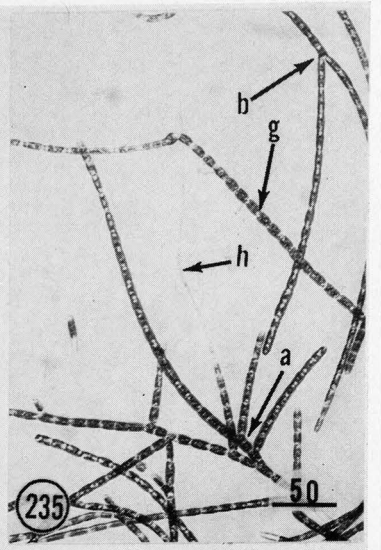
234


b
g
h
a
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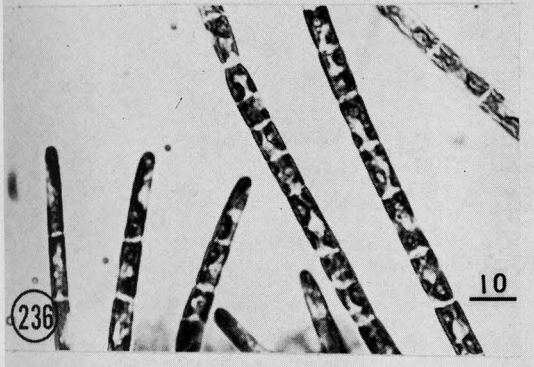
235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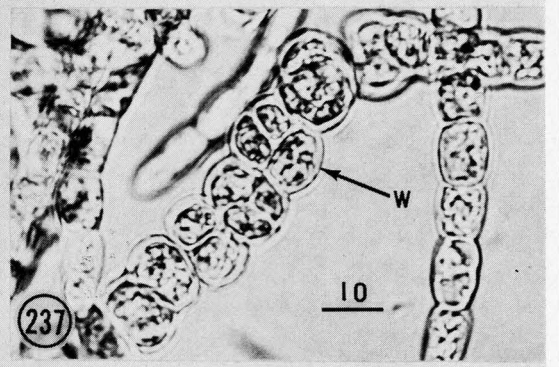
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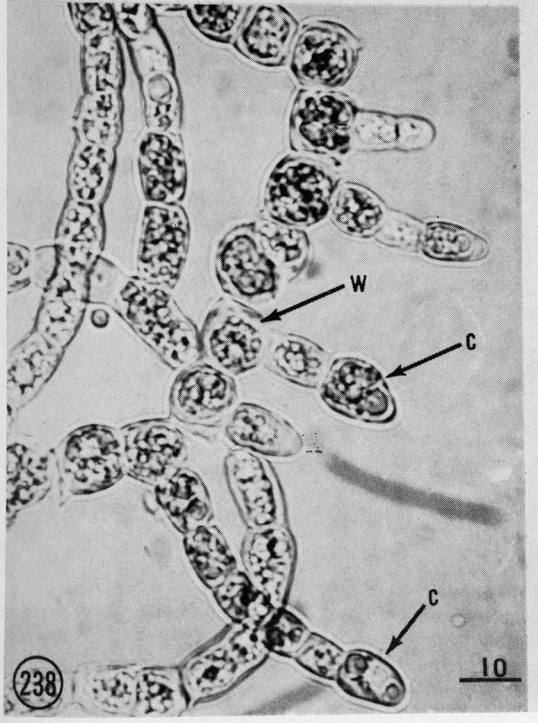
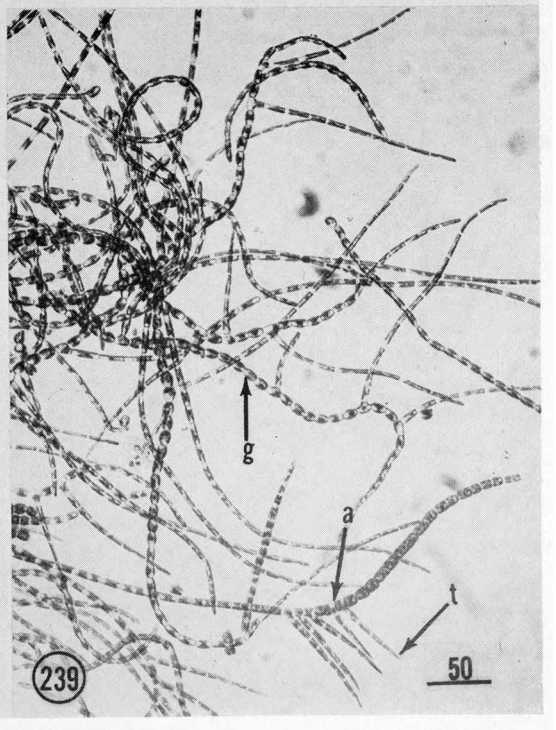

w
c
c
10
238


g
a
t
50
239

FIGURES 240–245

***Stigeoclonium farctum* Berthold**
(Size scale in microns)

Fig. 240–245. Variation in the morphology of the erect filaments in degree of branching, branching pattern, and branch tips:

Fig. 240. Unbranched erect filaments; pointed or acute branch tips (t).

Fig. 241. Unbranched erect filaments; approximate (a) and secund branching.

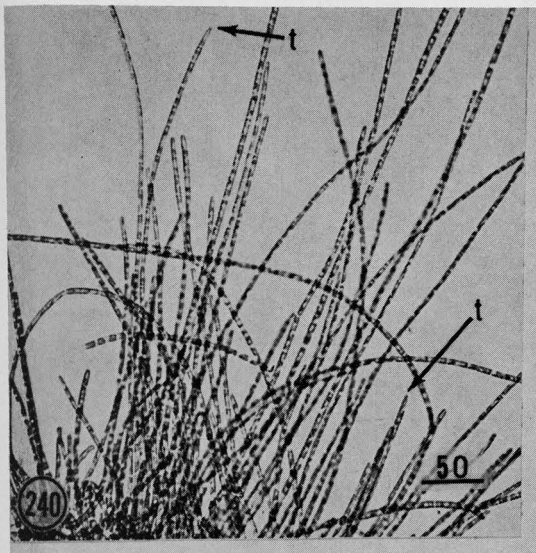
Fig. 242. Erect filaments irregularly, but not profusely, branched.

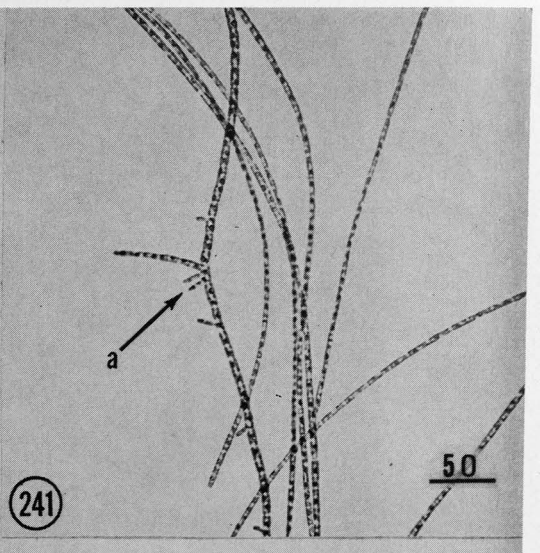
Fig. 243. Unbranched erect filaments forming zoospores (zf); erect filaments terminating in colorless hairs (h).

Fig. 244–245. Profusely and irregularly branched erect filaments; formation of downward-growing rhizoids (r) from cells of main filament.

Conditions of culture

Fig. 240–245. Isolate 19–5–V; BBMPTB$_{12}$ aerated wtih 2–5 % CO_2 in air; 3 weeks after inoculation.


t
t
50
240


a
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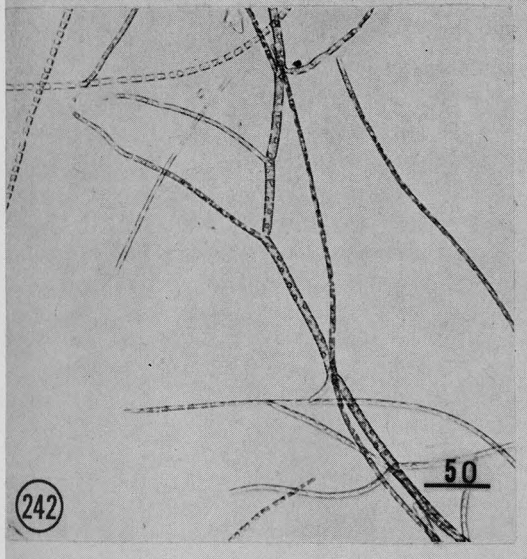
241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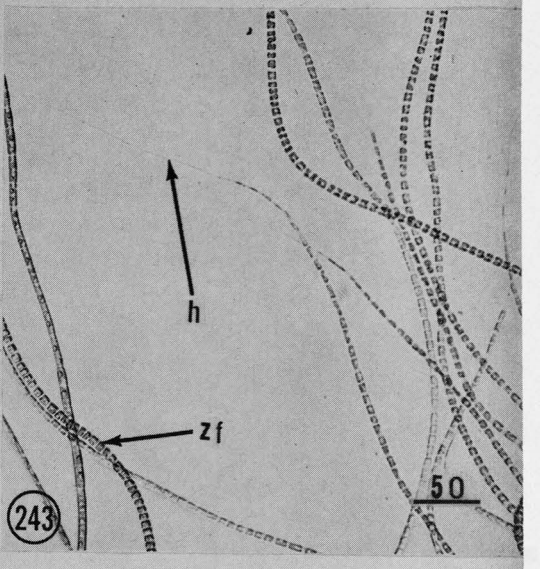
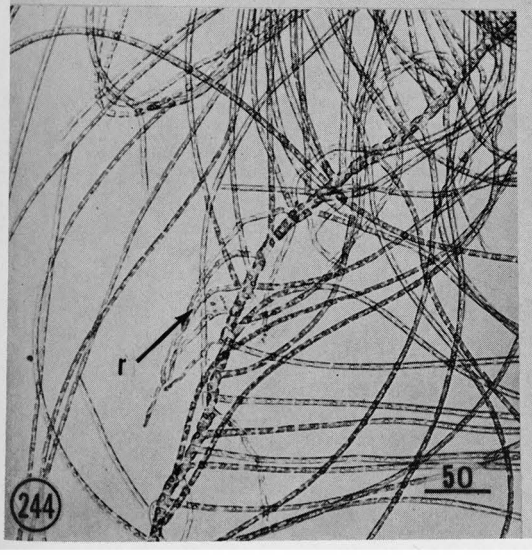
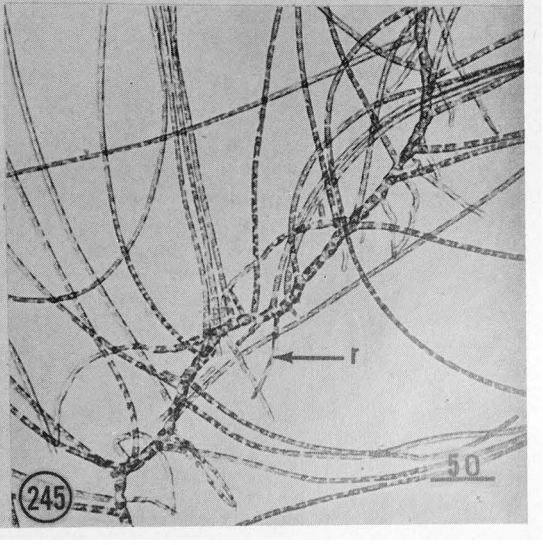

h
zf
50
243


r
50
244


r
50
245

FIGURES 246–248

***Stigeoclonium farctum* Berthold**

Fig. 246–247. Small, discrete colonies with distinct *Schizothrix*-like tufts (× 24).

Fig. 248. Large colony covered with distinct tufts (× 24).

Conditions of culture

Fig. 246. Isolate 19–5–V; 1.5 % $BBMPTB_{12}$ agar; 1 month after inoculation.

Fig. 247. Isolate 5–3F; 1.5 % $BBMPTB_{12}$ agar; 1 month after inoculation.

Fig. 248. Isolate 7–17; 1.5 % $BBMPTB_{12}$ agar; 1 month after inoculation.


246
247
248

FIGURES 249–256

***Stigeoclonium* sp.**
(Size scale in microns)

Fig. 249. Typical young germling. Branching filamentous prostrate system; colorless hairs produced from basal calls (h).

Fig. 250. Small, branching filamentous basal system; extensive erect system; erect filaments mostly unbranched; some alternate branching (b).

Fig. 251. Typical old culture. This organism was difficult to maintain in any liquid medium. After 2–3 weeks the zoospores were released without induction and the culture would form many young germlings, such as these.

Fig. 252. Filamentous basal system with proliferating lateral branches at end of filament.

Fig. 253–254. Zoospores (z) escaping from cells of erect filaments through small lateral pore in wall; erect filaments terminating in long, multicellular colorless hairs (h).

Fig. 255–256. Starch-filled, akinete-like cells. Cells of filaments tend to dissociate.

Conditions of culture

Fig. 249. Isolate S-5; $BBMPTB_{12}$; 4 days after inoculation.

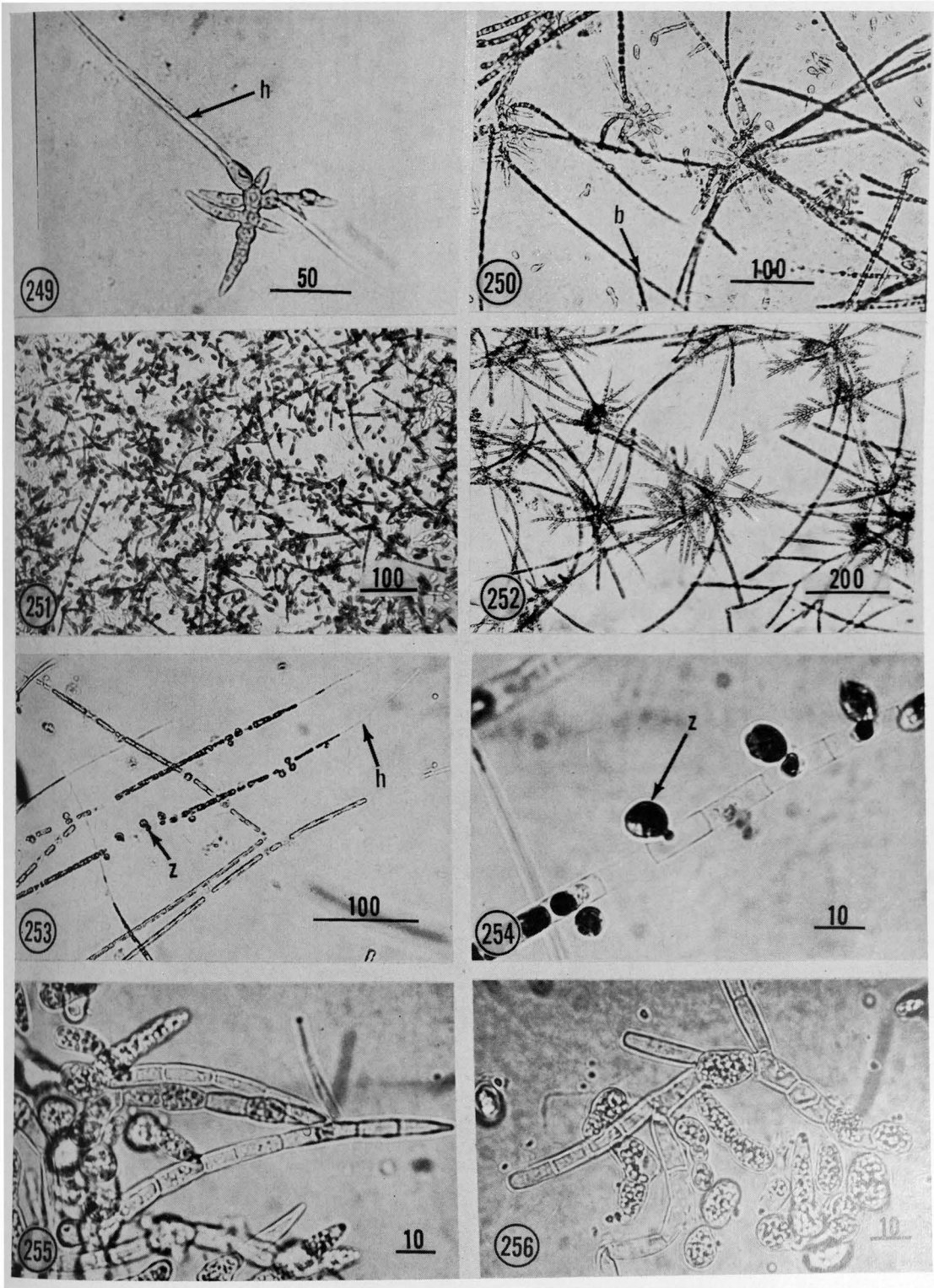
Fig. 250. Isolate S–5; $BBMPTB_{12}$; 2 weeks after inoculation.

Fig. 251. Isolate S–5; $BBMPTB_{12}$; 1 month after inoculation.

Fig. 252. Isolate S–5; 3 BBMP:1 SS; 2 weeks after inoculation.

Fig. 253–254. Isolate S–5; $BBMPTB_{12}$ aerated with 2–5 % CO_2 in air; 1 week after inoculation.

Fig. 255–256. Isolate S–5; 1.5 % $BBMPTB_{12}$ agar; 2 months after inoculation.


h
249
50
b
250
100
251
100
252
200
h
z
253
100
z
254
10
255
10
256
10

FIGURES 257–259

***Stigeoclonium* sp.**

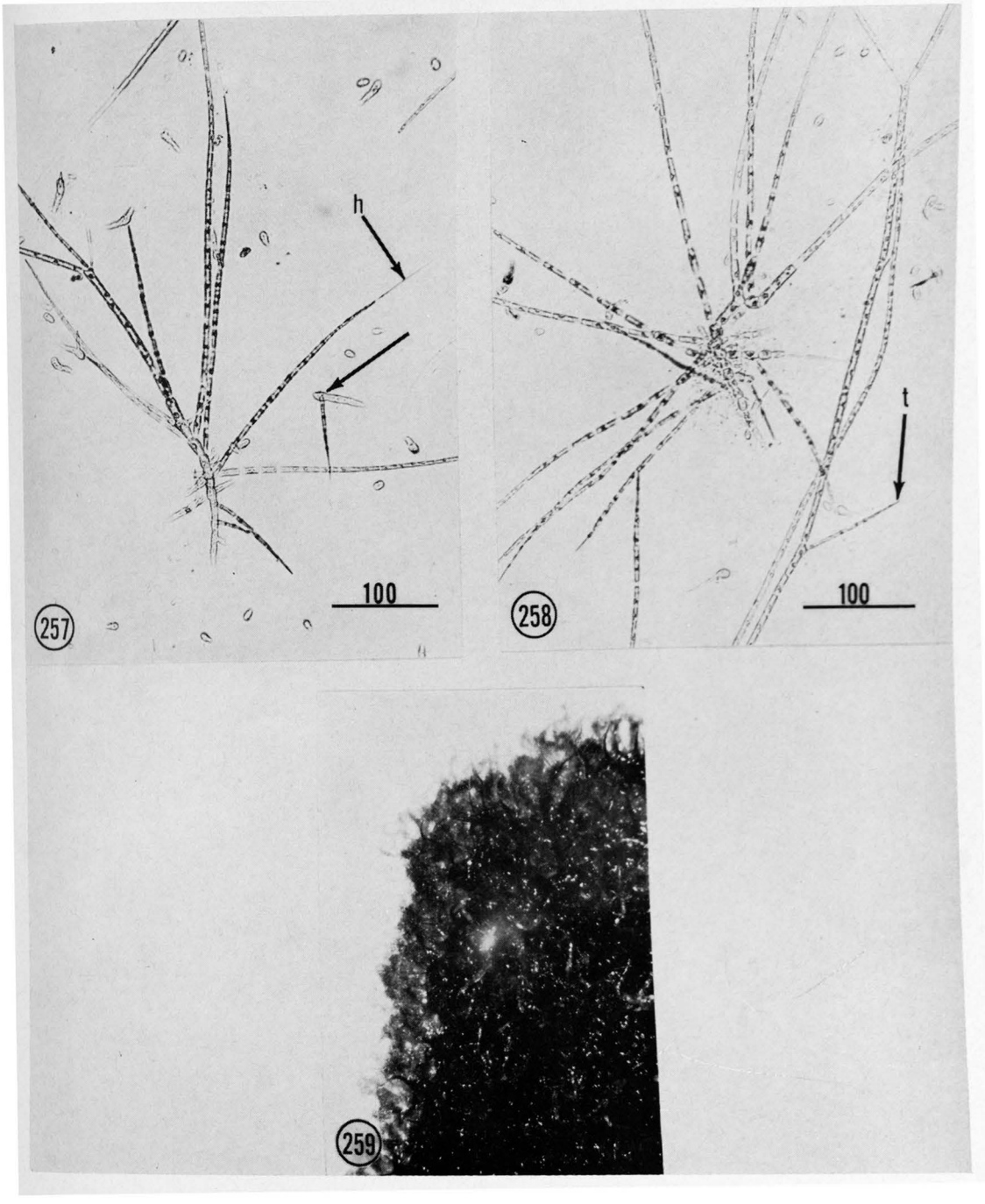
Fig. 257–258. Small, filamentous basal system; alternate-dichotomous branches ending in long, multicellular colorless hairs (h) or sharp points (t); germinating zoospore—type I (arrow). (Size scale in microns.)

Fig. 259. Tufted or matted colony, edge of curved bundles of filaments (× 24).

Conditions of culture

Fig. 257–258. Isolate S–5; $BBMPTB_{12}$; 2 weeks after inoculation.

Fig. 259. Isolate S–5; 1.5% $BBMPTB_{12}$ agar; 1 month after inoculation.


h
100
257
t
100
258
259